JOURNAL OF DAIRY SCIENCE

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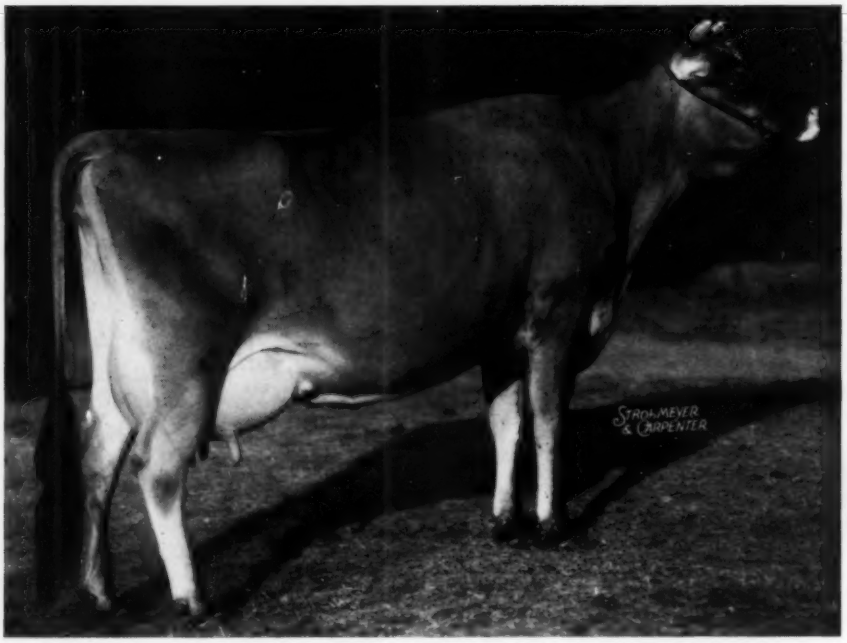

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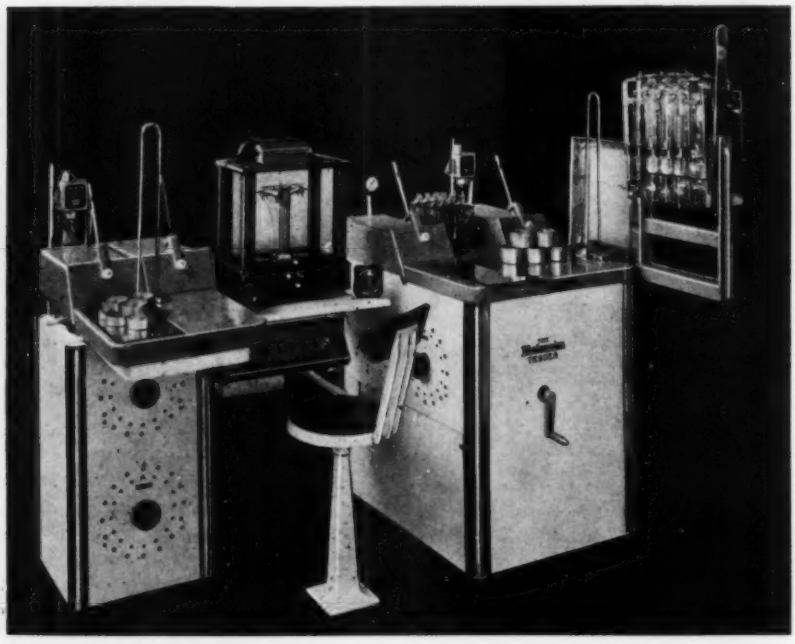

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THE RÔLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS. I. MILK PROTEINS

M. S. EL-RAFEY[1] AND G. A. RICHARDSON

Division of Dairy Industry, College of Agriculture, University of California, Davis

INTRODUCTION

The study of the foaming problem is of considerable interest in the dairy industry as well as several other industries. Some practical problems such as churning of cream, shrinkage of ice cream, whipping of cream or condensed milk, prevention of milk solids losses in manufacturing processes, foaming of casein in paper-coating, and the efficiency of pasteurization are directly related to the foam problem.

The surface-active constituents of milk contributing to its foaming properties, in all probability, are involved also in other physico-chemical problems such as creaming of milk, rebodying of cream, insolubility of milk powders and in the various activities at the fat globule interface.

A survey of the literature indicated the desirability of undertaking a systematic study of the fundamental foam problem in milk products. In the present paper, the apparatus used in these studies and the units of foam measurements are described, and the foaming properties of the major milk proteins are reported.

REVIEW OF LITERATURE

At the beginning of the present century, Siedel and Hesse (40) presented quantitative proof of a protein accumulation in the milk foam. This work, confirmed later by others (20, 38) marked the start of the present state of confusion as to what protein is responsible for the foaming of milk. Siedel (39) introduced the notion that the foaming substance in milk is other than its two major proteins—casein and albumin. Subsequent efforts were directed towards the finding of the foaming substance in the minor fractions of milk proteins.

Rahn (31) claimed that milk contains a special foam compound, which is probably the protein that surrounds the fat globules. This suggestion did

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not clarify the question to any extent. The protein of the stabilizing film on fat globules in milk has been identified as (a) albumin, (b) glycoprotein, (c) globulin-like protein, (d) new protein, (e) casein and (f) a mixture of proteins (6).

Hekma and Brower (15) concluded from microscopical studies that the separator slime came from collapsed foam cells. Grimmer and Schwartz (13) studied the nature of the foaming material in milk by analyzing the separator slime, and found that it contained only 36 per cent casein in the protein fraction. The other 64 per cent protein was claimed to be a new protein probably identical with the foaming substance of milk. Rahn and Sharp (32) called this substance "Schaumstoff."

Ansbacher, Flanigan and Supplee (1) eluted a protein fraction from high-foaming commercial casein. The eluted casein lost its foaming ability, whereas the elution product showed remarkable foaming. There was no definite conclusion as to the identity of this foaming substance, but a globulin with a sterol prosthetic group was suggested. According to the authors, the foaming compound can be separated from milk after removing all the major proteins of milk.

Sharp, Myers, and Guthrie (38) analyzed separated milk foam for the protein fractions. They concluded that there is no preferential accumulation of any major protein fraction in the foam. Sharp and Krukovsky (37) reported the isolation of an agglutinin from milk, which is responsible for the clustering of solid fat globules and the creaming of milk, and also for the foaming of skim milk separated at 50° C.

The modern conception of the factors and forces governing different stages in the existence of a foam will not be reviewed because they have recently been reviewed by Berkman and Egloff (3, 4).

The present investigation represents an attempt to establish a better understanding of the rôle of milk proteins involved in the foaming of milk and some of its products. It includes the isolation and study of the foaming properties of casein, lactalbumin and lactoglobulin.

EXPERIMENTAL

The foam apparatus. The foam measuring apparatus used in the present work is shown in figure 1. It is a modification of the type used by Hansley (14) and others (5, 8, 12, 18). The chief improvement is the provision for separating the foam from the liquid. A known volume of air is forced at a measured pressure through a sintered glass disc into the layer of liquid above it. The volume and the stability of foam which is formed are measured at the desired temperature.

Foam measurements. In standardizing the apparatus, the most reproducible results were obtained with separated milk under the following conditions: 1) volume of sample, 50 ml., 2) time of forcing air through the

sample, 20 seconds, 3) manometer pressure, 30 mm. of mercury and 4) reserve pressure on storage tank, 5 pounds per square inch (0.35 kg./cm.^{2}).

The volume of air that passes through the sample varies linearly with the temperature from 195 cc. at 5° C. to 270 cc. at 55° C. with a range of ± 2 per cent.

To determine the comparative quality of a foam, the sample was tempered in the water bath at the desired temperature and two measurements were obtained. 1) The foam height which is the reading taken immediately after shutting off the air pressure. 2) The average duration of half the

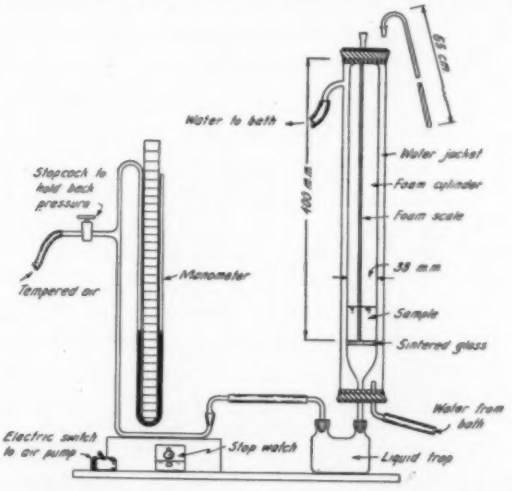

FIG. 1. Foam apparatus. The apparatus consists of a glass cylinder tapered at the lower end to form a ground glass joint. A sintered glass plate is sealed into the tube above the tapered portion. The upper end is sealed to a ground glass joint which permits a connection of a bent glass tube. The cylinder is surrounded by a removable water jacket connected to a pump that circulates the water from a thermostatically controlled water bath. The air pressure is generated by an air compressor provided with a storage tank, a gauge to indicate air storage pressure and a needle valve to control air volume and hence the working air pressure. The temperature and humidity of the air are controlled. A capillary tube mercury manometer connected to the air circuit indicates the pressure to the disc.

volume of the original foam produced, expressed in seconds. The latter is a measure of the foam stability and is termed "half-volume time."

The half-volume time: The introduction of the half-volume time unit proved to be of value in the present study where the stability of a static foam is to be compared with that of dynamic foam, and where the foams do not obey the same subsidence equation. The mathematical equations to calculate the foam life proposed by Lederer (18), Bikerman (5) and Ross and

Clark (8, 35) are not applicable to milk at all temperatures. Figure 2 shows the rate of collapse of raw separated milk foam (0.03 per cent fat) at different temperatures (the time factor in this figure is on a logarithmic scale). It is quite evident that no one formula can be used to calculate the average life of a bubble at all these temperatures. There is an apparent rest period for the separated milk foam before it starts to break down—after which the foam may either gradually subside or explode partially or completely. The "half-volume time"[2] was found to be the best measurement for comparing these foams. It has the advantage over reading the foam height after a certain time interval, which has been used in research on milk foams (19, 22, 36) in that it shows the relative stability of foam that sub-

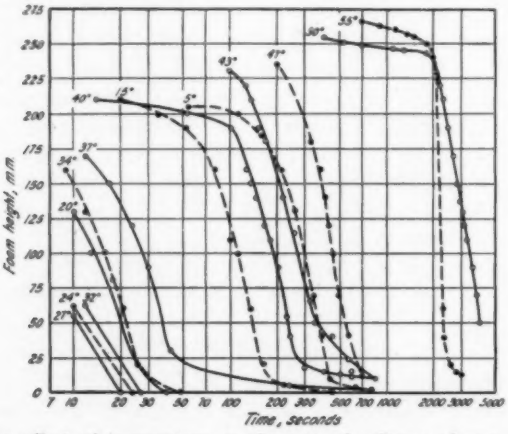

FIG. 2. The effect of temperature on the rate of collapse of raw skim (0.03 per cent fat) foam.

sides completely before the time interval or remains unchanged after the chosen time. This measurement, however, has the disadvantage of being time-consuming and requires constant observation of the foam until it breaks to half its volume. In order to minimize this disadvantage and still get comparable results, an upper limit for the stability was set. This limit was selected as 3,000 seconds, which is approximately the highest value obtained for milk and separated milk foams within the limits of temperature (5 to 55° C.) chosen in this study. The half-volume time of the samples studied at different temperatures seems to be independent of the foam height. This unit can be readily converted to Σ, the average life of a foam bubble, in case of logarithmic subsidence, using the equation $\Sigma = \frac{\text{half-volume time}}{2.303 \log 2}$.

[2] After completion of this work, a somewhat similar unit of measuring the foam stability was used by F. Schultz (Trans. Faraday Soc. 38: 85–93, 1942) which is called "foam-time." An excellent account of this unit of foaminess is presented in his paper.

Other measurements. The surface tension measurements were made by a Cenco-du-Noüy interfacial tensiometer. The readings were made within one minute after the surface had been renewed by stirring. Viscosity determinations were made by the Ostwald viscosimeter. For the determination of nitrogen a semi-micro Kjeldahl distillation apparatus, described by Redemann (33) was used, and 30 per cent hydrogen peroxide was added to aid the final oxidation. The pH values were checked with a quinhydrone electrode using a saturated calomel cell and a gold electrode.

ISOLATION OF MILK PROTEINS

Casein. A survey of the literature (9, 16, 34, 43, 44, 46, 47) shows that there is no method available to separate casein from milk which would be suitable for physico-chemical studies, if the results are to be interpreted in terms of the casein as it exists in milk. Several attempts were made to devise a procedure for isolating casein which is very low in fat and in a non-denatured form. The following was found to be a very suitable procedure: Raw skim milk, separated at 32° C., cooled to room temperature, was passed twice through a Sharples supercentrifuge at 36,000 rpm. The milk fat content of the skim milk as determined by the Babcock method was thus reduced from 0.015 per cent to 0.01 per cent to 0.00 per cent successively. The protein concentration decreased from 3.3 per cent to 2.8 per cent. The supercentrifuged skim milk was held overnight at 2° C. and the casein was precipitated by adding 0.1 N HCl according to the recommendations of Van Slyke and Baker (47). After complete sedimentation of the casein, the whey was filtered off by suction in a large Buchner funnel over filter paper. The casein then was washed thoroughly twice with cold distilled water acidified with HCl to pH 4.6, and three more times with cold distilled water. The casein was filtered by suction after each washing, then the moist casein was spread out in a glass dish and placed in the ice room at – 10° C. overnight. The frozen casein was then dehydrated with the aid of an electric fan. After three successive freezings and dehydrations, the semi-dry casein contained about 41.5 per cent total solids and 40.2 per cent protein. The fat content as determined by the Mojonnier method was 0.09 per cent calculated on the dry basis. The casein obtained was in the form of white fluffy particles which were easily soluble in calcium hydroxide and had a very high foaming capacity.

Calcium caseinate solutions were prepared according to Palmer's method (27) with a slight modification. The casein was ground with sufficient calcium hydroxide powder to bring the solution to pH 6.6 (instead of pH 8.0 and subsequent neutralization to pH 6.6 with phosphoric acid). The concentrated casein solutions (about 3.3 per cent) thus obtained were diluted with distilled water and phosphate buffer solution (Sorensen) to give the desired concentration.

Lactoglobulin. The lactoglobulin fraction in separated milk is about 0.5 grams per liter. It is obviously desirable, therefore, to employ some simple means of concentrating the whey before submitting it to procedures involving salting out and dialysis. Palmer and Richardson (28) and Palmer (26), described a simple and safe method by which the whey was concentrated by freezing. The concentrated whey (3:1) thus obtained was half-saturated with 18 grams of anhydrous sodium sulfate per 100 ml. of whey and left for eight hours to precipitate at room temperature (the brownish white precipitate concentrated at the surface of the container). The lactoglobulin was filtered, washed with half-saturated solution of sodium sulfate, dissolved in distilled water, dialyzed against ice water and then against 3 per cent sodium chloride. The solution further concentrated by pervaporation (17), was stored in sterilized bottles at 2° C. and was used within 10 days of its preparation. The interference of the fat with the foaming of lactoglobulin led to the use of supercentrifuged rennet whey or the acid whey obtained from supercentrifuged skim milk. It was found necessary also in some instances to extract the concentrated lactoglobulin solution with hexane.

Lactalbumin. The whey filtrate, after removing the globulin fraction, was warmed to 35° C. and sodium sulfate added to bring its final concentration up to 36 grams salt per 100 ml. (26). The white precipitate filtered off at 35° C. was dissolved in distilled water and dialyzed against running water at 2° C. until free from sulfate ion. The crystal clear lactalbumin solution was further concentrated by pervaporation and stored in a sterilized bottle at 2° C. for use within 10 days of its preparation. According to reports by Pedersen (29), one can assume that the lactalbumin thus obtained was composed of the α and β fractions.

RESULTS

Foaming Properties of Casein

Effect of concentration. The effect of a progressive increase in concentration of casein on the foaming stabilities of its solutions is shown in figure 3. In both the presence (2.7 per cent) and the comparative absence of fat (0.06 per cent dry basis), it is to be observed that with increasing concentration of protein, the stability of the foam increases to a maximum then decreases again. The foam heights (50) on the contrary, were observed to reach a maximum value with no further decrease with increasing concentrations. The surface tension decreases to a constant value. In these experiments, the pH of the solutions was kept at 6.6 by the use of a phosphate buffer. The zone of maximum foam stability seems to be a function of the amount of fat present in the solution and the temperature at which the foaming properties are tested. Low temperature and low fat content favor the stability of calcium caseinate foam.

Effect of temperature. Preliminary results indicated that the foam height, foam stability and surface tension of calcium caseinate solutions all decreased progressively as the temperature increased from 5 to 55° C. The greatest effect of temperature was found to be on the stability of the foam. Casein prepared by the Hammarsten or Van Slyke and Baker methods did not give stable foams which might be due to partial denaturation of the protein during the method of preparation. As an example, the half-volume time of the foams of 2.8 per cent calcium caseinate solution (Van Slyke and Baker methods) was 310 seconds at 5° C. and 13 seconds at 55° C. Casein prepared by Cohn and Hendry's method contained 2.7 per cent fat on the dry basis (Mojonnier). The foam stability of this casein was low due to the interference of fat; the half-volume time of 2.4 per cent calcium

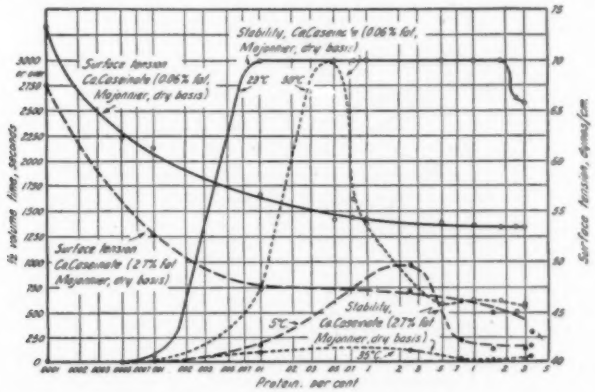
Stability, CaCaseinate (0.06% fat, Mojonnier, dry basis)
Surface tension CaCaseinate (0.06% fat, Mojonnier, dry basis)
23°C
55°C
Surface tension CaCaseinate (2.7% fat Mojonnier, dry basis)
Stability, CaCaseinate (2.7% fat Mojonnier, dry basis)
5°C
55°C
½ volume time, seconds
Surface tension, dynes/cm
Protein, per cent

FIG. 3. The effect of concentration on the foam stabilities and surface tension of Ca caseinate solutions.

caseinate solution was about 220 seconds at 5° C. and 17 seconds at 55° C. It was also observed that elution of casein by Ansbacher, Flanigan and Supplee's method (1) did not decrease the foaming capacity or the stability of the calcium caseinate solution. In some cases, an actual increase with the eluted casein was observed which was correlated with a decrease in the fat content of the casein. Figure 4 shows a sample of the results obtained.

Calcium caseinate solutions prepared by the new method described in this paper from ultracentrifuged separated milk yield foams of greater capacity and stability (figure 5). Our data have shown that the surface tension of a 1.1 per cent calcium caseinate solution decreases only slightly with an increase in temperature (from 54.2 dynes at 5° C. to 50 dynes at 55° C.) and the foam heights are approximately proportional to the volume of air introduced at all temperatures (50). The greatest change is observed in the stability measurement, which increases slightly with temperature up to

22° C., then starts to decline slowly at first up to 35° C., and rapidly afterwards to 55° C. This great improvement in the stability of the foam is doubtless related to the lack of denaturation of the protein as well as the low fat content of casein prepared by this method.

Effect of fat. The addition of 0.015 per cent milk fat emulsion to the calcium caseinate solution decreases the foam height and the stability of the

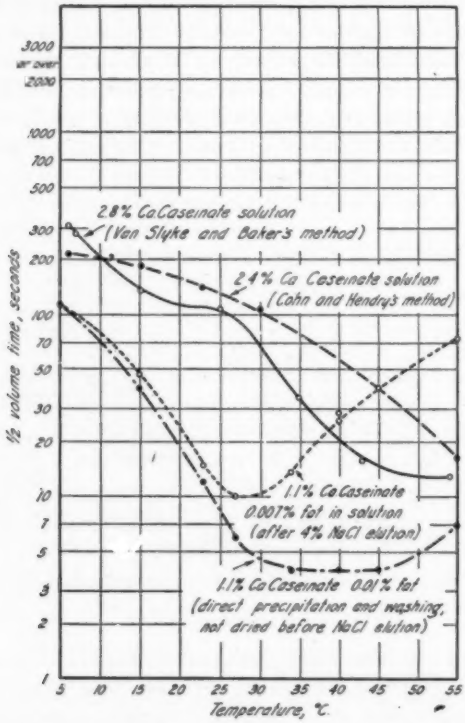

FIG. 4. The effect of methods of preparing casein on the foam stabilities of caseinate solutions.

foam to a marked extent. The reduction of the stability of the foam is greatest at temperatures above 15° C. as shown in figure 5.

The fat emulsion was prepared by emulsifying about 1 per cent milk fat in dilute phosphate buffer at 60° C. followed by cooling in ice water. The emulsion showed marked stability due to the decrease of the fat/solution interfacial tension by the salts.[3]

[3] See Harkins, W. D., Some aspects of surface chemistry fundamental for biology, Jour. Chem. Physics., 6: 171, 1938.

FOAMING QUALITIES OF LACTOGLOBULIN

Effect of concentration. Lactoglobulins prepared from supercentrifuged rennet whey do not give stable foams. The effect of increasing the lactoglobulin concentration from 0.0001 to 0.75 per cent on the foam stability was tested at 25° C. and 50° C. The results plotted in figure 6 indicate that the foaming capacity increases slightly with concentration at 25° C. The results obtained at 50° C. show that there is a subsequent decrease in foam

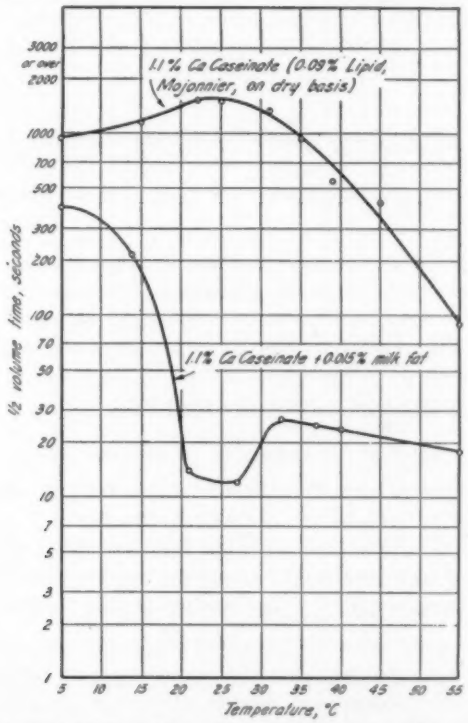

FIG. 5. The foam stabilities of Ca caseinate solutions of casein prepared by the present method and the effect of adding milk fat emulsion.

stability with an increase in concentration. The surface tension continues to decrease as the concentration increases. The foam stability follows the same pattern as that reported for calcium caseinate. On the whole, it is quite clear that lactoglobulin has very poor foaming qualities. This may be attributed to the presence of a lipid material associated with its protein molecule (10, 24).

Effect of temperature and fat content. Table 1 shows that 0.05 per cent lactoglobulin solutions (the concentration normally present in milk) do not

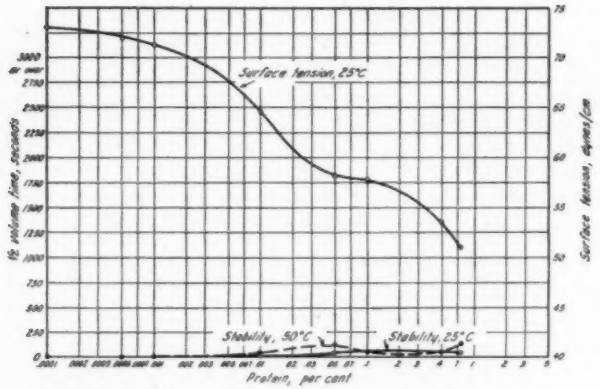

FIG. 6. The effect of concentration on the foam stabilities and surface tension of lactoglobulin solutions.

form stable foams at any temperature tested, when the solution contained about 0.006 per cent fat. As the fat content was decreased to 0.001 per cent by extracting the same solution twice with equal volumes of hexane, the foam height and the stability increase at all temperatures tested, the increase being greater at temperatures above 20° C. Lactoglobulin solutions were prepared in phosphate buffer solutions at pH 6.6.

THE FOAMING PROPERTIES OF LACTALBUMIN

Effect of concentration. The effect of increasing the concentration of lactalbumin at 25° C. and 50° C. is illustrated in figure 7. As the concentration increases from 0 to 1 per cent the foam stabilities increase to maximum values and remain unchanged (the foam heights were observed also to reach maximum values (50)). The surface tension continues to decrease

TABLE 1

Foaming properties of lactoglobulin. Effect of temperature on 0.05% lactoglobulin solutions of different fat contents

0.05% lactoglobulin precipitated once from rennet whey (0.0065% fat in the solution)				Same globulin solution extracted twice with hexane (0.001% fat in the solution)			
Temperature	Surface tension	Foam height	½ volume time	Temperature	Surface tension	Foam height	½ volume time
°C.	*dynes*	*mm.*	*sec.*	°C.	*dynes*	*mm.*	*sec.*
5.0	54.4	45	5	6	49.0	120	20
15.0	51.6	25	5	10	49.1	115	19
22.5	49.4	8	2	17	.....	100	17
32.0	48.2	40	4	20	48.0	120	21
43.0	47.5	190	20	32	49.3	220	50
52.0	47.1	260	72	40	46.9	260 .	380

to a minimum value. It is of interest to note that lactalbumin is more surface-active with regard to foaming, at 50° C. than at 25° C., as evidenced by the fact that less protein concentration is required to support a stable form at the former temperature.

Effect of temperature. The results illustrated in figure 8 show that a 0.047 per cent lactalbumin solution forms a much more stable foam than a corresponding concentration of lactoglobulin solution. The half-volume time increases with the rise of temperature from 5° C. to 23° C. where it reaches the maximum limits set for this study and remains so up to 50° C. When the protein concentration is increased to 0.18 per cent, the foam stability exceeds the limits at all temperatures. The foam volumes were found to be equal to the volume of air forced through the solutions (50).

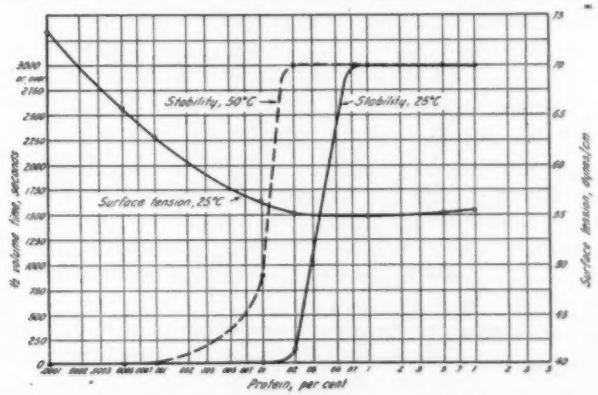

FIG. 7. The effect of concentration on the foam stabilities and surface tension of lactalbumin solutions.

Effect of fat. The effect of adding different amounts of milk fat emulsion stabilized in dilute phosphate buffer is quite interesting—figure 8. The addition of 0.0005 per cent fat to a 0.05 per cent lactalbumin solution decreases the stability of the foam greatly at temperatures below 25° C. and to a less extent at 30.5° C. but has no effect above 38° C. When the fat content is further increased to 0.005 per cent in 0.42 per cent lactalbumin solution, the reduction in the foaming properties is much greater at all temperatures. These changes are accompanied by a lower surface tension. A further increase of the fat content to 0.5 per cent in the 0.05 per cent lactalbumin solution, decreases the foam measurements (and surface tension (50)) to still lower limits. It is clear then that the foaming capacity of a lactalbumin solution is a function of the amount of fat present and the temperatures at which the foaming properties are measured. The inhibiting effect of milk fat is reduced at temperatures above its melting-point. The

next experiments were designed to study the effect of increasing the albumin content in a solution, the foaming properties of which were greatly inhibited by the presence of a relatively high fat concentration. As shown in figure 8, increasing the concentration of albumin from 0.05 to 0.5 per cent in a solution containing 0.5 per cent milk fat improves the stability of the foam only slightly at temperatures below 39° C. but to a great extent above that tem-

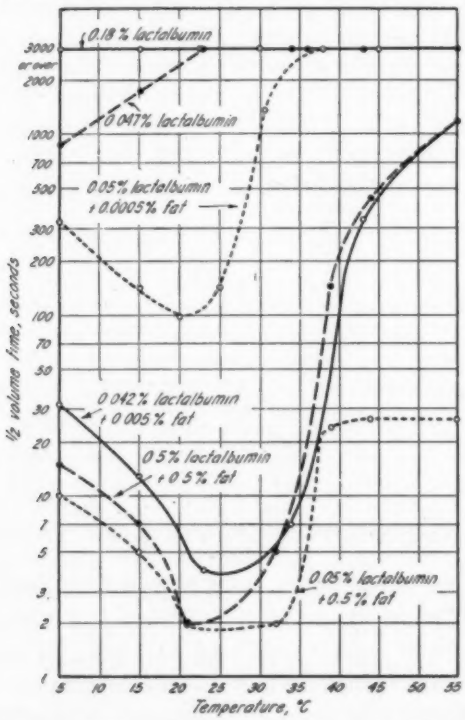

FIG. 8. The foam stabilities of lactalbumin solutions with different amounts of milk fat emulsion added.

perature. The surface tension values are about the same in both solutions (47.3–47.0 dynes respectively (50)).

DISCUSSION

The experimental results have shown that the foaming properties of solutions containing surface-active constituents of milk vary markedly with a number of factors depending on the chemical nature and the amount of materials present in the solution, and the physical conditions under which the measurements are obtained. The variations in the foaming properties

of milk proteins can be discussed best if only the stability of the foam is considered, since in some instances the foam heights are equal while the stabilities are quite different.

The effect of increasing the concentration of calcium caseinate or lactoglobulin in buffered solutions at pH 6.6 results in a progressive increase in the foam stability to a maximum followed by a subsequent decrease. The latter decrease is not noticed in the case of lactalbumin solutions up to 1 per cent concentration. The explanation of these facts cannot be based on changes of surface tension[4] or viscosity, a conclusion which has been reached by several investigators (11, 23, 30, 49). The same phenomenon of the relation of concentration to the foam stability, observed by Bartsch (2) on simple organic compounds, was explained on the boundary heterogeneity theory. Thus, the foam stability increases when the air/solution interface becomes heterogeneous with respect to the solvent and the solute (a condition which is not present in very dilute or concentrated solutions). Talmud (45) relates the maximum foam stability to the concentration that allows for maximal hydration at the interface. At the optimum concentration the organic molecules are oriented, inclining at a certain angle to the surface and interlacing with each other to form a kind of gel structure or a film with the highest tensile strength. Therefore, in order to discuss the stability of protein foams, consideration should be given to the micellar size, solubility, orientation, and the degree of hydration, when such information is available. Lactalbumin, being highly soluble in water and having a low molecular weight (17,500–39,000) (29) might well be placed with the soluble compounds in Bartsch's classification of the foaming substances (2). On the other hand, casein and lactoglobulin with a low solubility in water and with high micellar weights (average 87,000 and 140,000 respectively) (29) may be placed with insoluble substances in the same classification.

The effect of temperature on the foam stability of milk protein solutions may be explained on the same principle; *i.e.*, there is an optimum molecular size and degree of hydration that permits the formation of a lamella which has a high tensile strength. For example, a solution of calcium caseinate (figure 5) forms a foam that has a half-volume time of approximately 1520 seconds at 22° C. and 90 seconds at 55° C. This rapid decrease in foam stability at temperatures above 35° C. can be correlated with the fact observed by Svedberg, Carpenter and Carpenter (44) that the micellar size of calcium caseinate is doubled by warming its solution to 40° C. These authors believe that the heating causes a polymerization or association of the molecules to form larger aggregates. Such an increase in size along with the dehydration of casein upon heating (23, 48) would seem to be among the important

[4] K. G. A. Parkhurst, in a recent paper (Trans. Faraday Soc., 37: 496–505, 1941), stated that it is not low surface tension *per se* that ensures foam stability but the nature of the adsorbed layer giving rise to the lowering of surface tension.

factors responsible for the decrease of foam stability of a calcium caseinate solution heated to 55° C. Further evidence on the importance of the particle size of calcium caseinate to the foam stability of its solution can be obtained from correlating the findings of Carpenter (7) with the foam stability obtained in this study. According to Carpenter, the molecular weight of casein in a 1.5 per cent solution is 96,000 at pH 6.8 (M/60 phosphate buffer), but on dilution to less than 0.7 per cent, the micelle dissociates to one-third the initial weight, namely 32,000. This dissociation is completely reversible and thus confirms Sorenson's conception (41, 42) of a protein as a complex undergoing reversible association and dissociation. This decrease in micellar size on dilution is accompanied by changes in physical properties, as for example, an increase in specific rotation. The foam stability of different concentrations shown in figure 3 indicates that as the micellar weight of calcium caseinate decreases by dilution, the foam stability increases. For example, at 50° C. the half-volume times are 630 and 1370 seconds, corresponding to concentrations of 1.0 and 0.1 per cent respectively. At a concentration of 0.05 per cent, a calcium caseinate solution (prepared from unaltered casein) gives an apparently clear solution that has about the same micellar size (7) and foam stability as a 0.05 per cent lactalbumin solution. Likewise, sodium caseinate appears to have a smaller micellar size than calcium caseinate (43b) and the results, not reported in this paper, show that it has higher foam stability.

It is quite important to note that none of the casein solutions, at concentrations above 0.5 per cent, supports a stable foam at temperatures above 43° C., where the skim milk forms a very stable foam.

No data are available on the changes of particle weight or the degree of hydration of lactalbumin or lactoglobulin as the temperature is increased from 5 to 55° C. The facts reported in table 1 and figure 8 indicate that both protein solutions have greater stability at higher temperatures and, by analogy, it may be reasoned that the conditions favorable for forming lamellae with higher tensile strength and greater stability are better fulfilled at these temperatures. One of the present trends of thought in protein chemistry (21) suggests that the larger protein molecules consist of small primary protein units linked into a secondary structure by means of carbohydrate, phospholipid, nucleic acid or polyvalent inorganic ions, and that these units are subject to association and dissociation under different conditions (7, 21, 41, 42). It may be of value to consider the foaming stability as one of the physical properties that reflects such an association and dissociation as pointed out in discussing the foaming properties of calcium caseinate solutions.

The effect of adding small amounts of milk fat emulsion in phosphate buffer to milk protein solutions is quite interesting. The data indicate that the foam stabilities of lactalbumin solutions are reduced to a greater extent

by the fat at temperatures below the melting point of the milk fat (figure 8). The reverse is observed in calcium caseinate solutions (figure 5). A 0.05 per cent lactoglobulin solution does not give a stable foam in the presence of as little as 0.0065 per cent fat, yet the stability of the foam is greater at temperatures beyond the melting point of milk fat (table 1). The action of milk fat on the foaming properties will be discussed in a later paper.

SUMMARY

An apparatus is described for accurate measurements of the foaming properties of milk. Experimental data have revealed that the subsidence of skim milk foams cannot be defined by a single equation at temperatures between 5 and 55° C. For this reason, a unit called a "half-volume time" has been proposed and used to compare the stabilities of static and dynamic foams. The unit can be converted by a given equation to give average life of a foam bubble, if the foam follows a logarithmic subsidence.

The major surface-active proteins of milk, including casein, α-β-lactalbumin and lactoglobulin, have been separated, purified and studied for their foaming properties. A new procedure for isolating undenatured casein with a lipid content as low as 0.06 per cent has been introduced. The method is based on super-centrifuging skim milk, precipitating the casein at a low temperature with 0.1 N HCl, washing, freezing, and dehydrating the frozen casein by pervaporation.

The foam "half-volume time" values of milk protein solutions in M/30 phosphate buffers at the normal hydrogen ion concentration of milk varied between 2 and 3000 seconds (or over), depending upon the kind of protein, concentration, method of isolating the protein, temperatures, and the amount of milk lipids present in association with the protein molecule or added in the form of an emulsion to the solution. Studies of these variables are reported and some theoretical explanations advanced.

It is shown that calcium caseinate solutions of a concentration equivalent to that of milk and prepared by the present procedure have high foam stabilities at temperatures below 40° C. Lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming. The foam depressing action of milk fat is shown to be greater at temperatures over 15° C. with calcium caseinate solutions and at temperatures lower than 35° C. with lactalbumin solutions.

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THE RÔLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS. II. WHEY, SKIMMED MILK, AND THEIR COUNTERPARTS

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REVIEW OF LITERATURE

The foaming properties of some milk products represent a unique colloidal phenomenon; that is, the foam stability decreases to a minimum, as the temperature increases, after which it again increases. The first report on the effect of temperature was made by Siedel (22). He found that the quantity of foam depends on the temperature of the milk and that the nature of the foam at low temperature is different from that at high temperature. Siedel's studies did not go beyond 35° C., but he noticed an inflection in the stability of the foam. Leete (11) and Sanmann and Ruehe (18) studied the effect of temperature on milk foam. Separated milk and milk foamed least between 20 and 30° C. according to the former, and at 27° C. according to the latter. Both investigators agreed on the increase in the foam at temperatures beyond 30° C. but disagreed on the temperature at which the maximum foaming appeared.

There has been considerable speculation as to the cause of this minimum foaming ability of milk. Rahn and Sharp (15) raised the question whether these kinks in the foam-temperature curve indicate the presence of a specific foam-producing material or of a stabilizing substance in the foam. Ansbacher and co-workers (1) reported that the foam-producing substance eluted from casein showed a critical agglomeration at the temperature of the minimum foaming. They did not present data regarding the foaming stability of this highly foaming constituent of milk. Leviton and Leighton (12) tried to explain this kink on the basis of the work of King (10), who showed that the portion of the surface of milk covered with a film of fat increased with a rise in temperature from 10 to 20° C. They postulated that the decrease in the foaming power might be explained on the basis of the increase in the tendency of the fat to spread as the temperature rose. No comment was made on the further increase of foam formation with increasing temperature.

Holm (9) explained the minimum foaming of milk as a result of variation in those properties of the solution which evidence themselves in viscosity

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changes. Davies (3) considered that the range from 20 to 30° C. represents conditions in which hydration is practically nil and the stabilizing factors, *i.e.*, the decrease in surface tension and denaturation of the proteins, have not come into play. Ansbacher *et al.* (1), however, found that the viscosity of their foam material was the same as that of water.

The authors are not aware of any published work on the foaming properties of whey.

In a previous paper (5a) the authors reviewed the existing controversy as to the foaming constituents in milk. Using the "half-volume time" for measuring the foam stabilities of the major milk proteins, it was found that calcium caseinate solutions have high foam stabilities at certain concentrations at temperatures below 40° C.; lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming properties. Foam-depressing action of milk fat was found to be greater at higher temperatures with calcium caseinate solutions, and at lower temperatures with lactalbumin solutions.

The present work deals with the preparation of synthetic whey and separated milk by combining the milk protein fractions in an attempt to duplicate the foaming properties of these products. As a result an explanation of the effect of heat on the foaming properties of whey, separated milk and milk is offered.

EXPERIMENTAL

The foam measuring apparatus, technique, and other methods used have been described in a previous paper (5a). Separated milk and rennet whey used in this work were obtained from the University Farm Creamery. The foaming properties of the solutions studied were compared at different temperatures between 5 and 55° C.

The foaming properties of whey and synthetic whey. The variation of the foam stabilities of various wheys with temperature is shown in figure 1. As the temperature increases from 5 to 55° C. the foam stability of the ordinary rennet whey (0.035 per cent fat) decreases to a minimum value between 22 and 32° C. and is followed by an enormous increase at higher temperatures. On combining solutions of lactalbumin and lactoglobulin separated from this whey, to give concentrations of 0.61 per cent and 0.05 per cent respectively in a phosphate buffer of the same pH as the whey, the foaming properties were found to be practically the same as the original whey. The type and general characteristics of the foam at low temperatures were identical with those of lactoglobulin solutions; those at temperatures above 32° C. resembled lactalbumin solutions.

In order to reduce the fat content of the whey and of the lactalbumin and lactoglobulin separated from it without the use of fat solvents the whey was supercentrifuged. The fat content of the whey was thereby decreased from 0.04 per cent to about 0.0001 per cent, and the protein decreased by

0.02 per cent. The foaming properties were greatly improved. A similar improvement in the foam height and stability were obtained with acid whey prepared from supercentrifuged skim milk (5b).

Solutions of lactalbumin and lactoglobulin (prepared from supercentrifuged whey), when combined to give the same concentration as in whey, give essentially the same foaming properties as the supercentrifuged whey itself (figure 1).

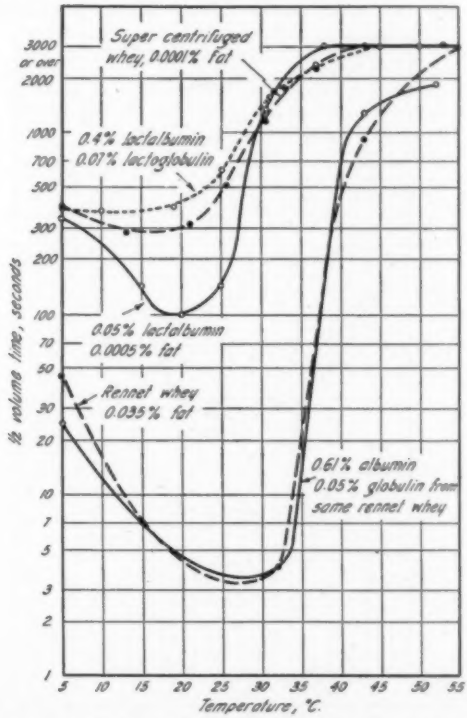

FIG. 1. The foam stabilities of whey with different fat concentrations and their duplication with lactalbumin solutions with fat or lactoglobulin added.

In a former paper (5a) it was shown that lactalbumin solutions (0.10 per cent or over) give stable foams represented by half-volume times greater than 3000 seconds at all temperatures. The present data indicate that the presence of lactoglobulin (from supercentrifuged whey) in a 0.4 per cent lactalbumin solution reduces the foam stability of the latter at lower temperatures to about 10 per cent of its original value. The addition of a milk fat emulsion in phosphate buffer to a lactalbumin solution (ratio 1:100 in solution), however, is shown to have an effect similar to the lactoglobulin.

On the other hand, when lactoglobulin, separated from ordinary rennet whey (0.035 per cent fat) is added to a 0.61 per cent lactalbumin solution, the foam stability of the latter is greatly reduced at the lower temperatures. The mixture has about the same foam stability as the original whey. The further reduction in this case undoubtedly is due to the presence of fat in the isolated lactoglobulin.

The foaming properties of separated milk. Raw separated milk (0.01 per cent fat by Babcock test) obtained from mixed herd milk forms a rela-

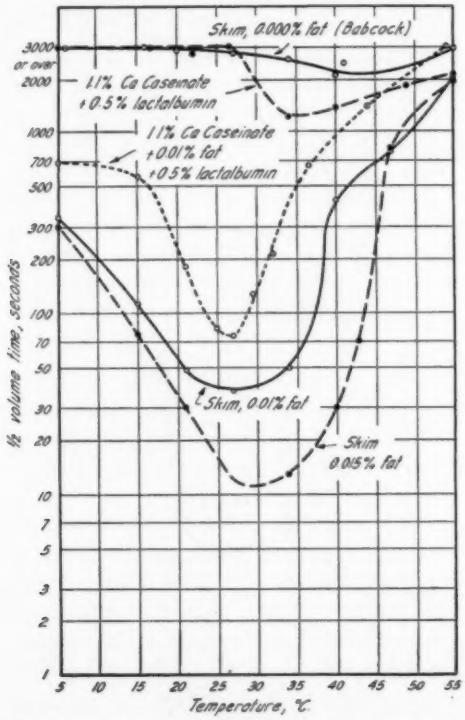

FIG. 2. The foam stabilities of skim milks with different fat content and their duplication with Ca caseinate + lactalbumin with and without milk fat added.

tively high stable foam at 5° C. On increasing the temperature to 25° C., both the foaming capacity and the half-volume time of the foam are reduced from 200 mm. and 429 seconds to 170 mm. and 21 seconds respectively. By increasing the temperature to 55° C. the foaming properties are improved again until a second maximum, much higher than the first, is reached (figure 2). The type of the foam changes also with the increase in temperature; that is, from relatively large and loose, to small, compact foam cells.

The effect of decreasing the fat content by passing the separated milk through a Sharples Supercentrifuge (36,000 rpm.) is shown in figure 2. As the fat content decreased from 0.015 to practically zero per cent (Babcock test), the skim milk showed remarkable foaming properties at all temperatures. The half-volume time of the foam at 27° C. increased from about 37 seconds (original) to about 2800 seconds (super-skimmed). There is no minimum foaming of supercentrifuged skim milk, which indicates that the

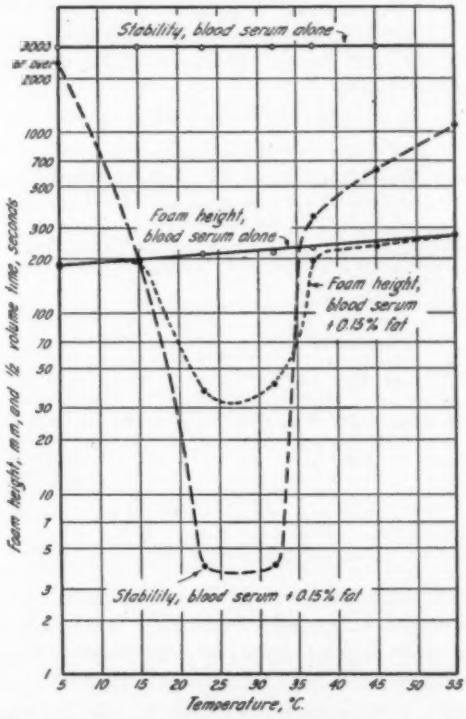

FIG. 3. The effect of adding fat emulsion on the foaming properties of "horse blood serum."

tendency of ordinary separator milk to give minimum foam values at room temperature is due to the presence of the fat globules.

Effect of adding fat emulsion to blood serum. To confirm further that the minimum foaming of separated milk is mainly due to the presence of fat globules, horse blood serum, obtained by defibrinating fresh blood with glass beads, was tested for foaming properties before and after the addition of a 0.15 per cent milk fat emulsion in phosphate buffer. The results reported in figure 3 show that the "half-volume time" is reduced by the presence

of fat from over 3,000 to 4 seconds at room temperature. Further increases or decreases in temperature improve both the foaming capacity and stability.

Comparison of the separated milk and whey foams. In comparing the foam-temperature curves of the separated milk (figure 2) and whey (figure 1) in the presence and absence of fat, it becomes evident that skim milk has a relatively higher foam stability in the temperature range from 5 to 35° C. than the corresponding whey. The main difference in the chemical composition of the skim milk as compared with the whey lies in the absence of casein from the latter. Therefore, it is reasonable to assume that the presence of calcium caseinate accounts for the greater stability of skim milk foam at lower temperatures.

Foaming of a reconstructed separated milk. On the basis of the previous findings that the constituents responsible for the foaming of the whey are lactalbumin and a lipid fraction, it was anticipated that by adding non-

TABLE 1

The effect of the addition of 0.05 per cent lactoglobulin on the foaming properties of a 1.1 per cent calcium caseinate (0.09 per cent fat, dry basis) solution*

Temperature	Surface tension		Foam height		Half-volume time	
	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin
°C.	*dynes/cm.*	*dynes/cm.*	*mm.*	*mm.*	*sec.*	*sec.*
5	54.2	54.6	201	200	935	850
15	53.7	52.8	213	215	1170	1030
25	51.0	51.6	220	218	1500	1525
35	51.0	50.6	247	241	950	595
45	50.2	49.4	245	235	423	182

* Prepared from twice supercentrifuged skim milk.

denatured calcium caseinate to these two constituents a solution would be obtained having the foaming properties of skim milk. The results obtained and shown in figure 2 confirmed that idea. It is possible to get an approximate duplication of the foaming characteristics of skim milk (0.01 per cent fat) with a solution containing 1.1 per cent calcium caseinate, 0.5 per cent lactalbumin, and 0.01 per cent milk fat emulsion in phosphate buffer. The increase observed in the foam stability of the synthesized solution can be accounted for by the absence of the phospholipid fraction which normally surrounds the fat globules. The effect of the fat globule membrane in determining the depressing action of the fat on the foam was previously studied (5). A buffered solution (pH 6.6) of 1.1 per cent calcium caseinate and 0.5 per cent lactalbumin gives approximately the same foam stabilities as the supercentrifuged skim milk (0.00 per cent fat by the Babcock test). Both the reconstituted and the supercentrifuged skim milk form foams of remarkable stability at all temperatures. The "half-volume times" of the foams were over 1000 seconds at every temperature used.

Table 1 shows that the addition of 0.05 per cent lactoglobulin to a calcium caseinate solution does not change the foaming properties of calcium caseinate appreciably except at the higher temperatures at which the fat is liquid. The lower foam stability of the mixture at 35° and 45° C. is to be expected in view of the additional lipid material contributed by the lactoglobulin.

From these results it is concluded that the foaming properties of skim milk are due to the presence of calcium caseinate, lactalbumin and milk fat. A solution containing 0.6 per cent lactalbumin, 0.05 per cent lactoglobulin, and 2.7 per cent calcium caseinate (which is prepared from casein extracted with hot alcohol, ether and dried) however, does not have the same foaming properties as skim milk (Table 2). The results support the belief that the physical properties of the casein treated with alcohol and ether are altered.

TABLE 2

Foaming properties of synthetic solutions containing casein which has been treated with alcohol and ether, in comparison to skim milk

No. Sample	30° C.		43° C.		55° C.	
	Foam height	½ volume time	Foam height	½ volume time	Foam height	½ volume time
	mm.	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*
1. 2.7% Ca caseinate solution (casein extracted with alcohol and ether and dried)	182	49	190	16	160	13
2. 2.8% Ca caseinate from casein No. 1, plus 0.6% lactalbumin and 0.05% lactoglobulin in solution	140	18	150	17	205	54
3. Skim milk + 0.018% fat	170	22	240	400	260	2005

The relation of "agglutinin" to the foaming of separated milk. Sharp and Krukovsky (19) attributed the high foaming properties of skim milk separated at 50° C. to the presence of an agglutinin and the poor foaming of that separated at 5° C. to the absence of agglutinin. This work is not in agreement with the present findings that calcium caseinate and lactalbumin are the chief foaming proteins in skim milk. Consequently, their work was repeated with the hope of finding the reason for such a difference in the foaming of separated milk obtained at 5° C. and 50° C.

Preliminary results obtained by separating raw milk at 5° C. and 32° C. indicated that the skim milk samples separated at 5° C. behaved abnormally with regard to the foaming properties; that is, the stability of the foam was reduced at 5° C. and at 55° C., but was increased at room temperature. Further research revealed that the resulting complications arose from two factors: 1) a slight development of rancidity which is probably due to centri-

TABLE 3

Effect of the temperature of separation on the foaming ability of the recombined milks

4.25 per cent pasteurized milk separated at two temperatures—at 5° C. (skim milk 0.11 per cent fat and 22.6 per cent cream) and at 53° C. (skim milk .015 per cent fat and 23.5 per cent cream). All the possible combinations of 4.25 per cent milks were prepared—the foaming properties of the original raw milk and the skim milks were tested at 3 different temperatures.

Sample	5° C.			25° C.			50° C.		
	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	dynes/cm.	*mm.*	*sec.*	*dynes/cm.*	*mm.*	*sec.*	*dynes/cm.*	*mm.*	*sec.*
(1) Raw milk	53.0	204	47	46.9	35	5	.....	270	1800
(2) Pasteurized milk	53.6	200	34	47.3	22	4	.....	288	3000
(3) 53° C. skim 0.015% fat	54.9	200	460	53.5	180	19	49.9	280	3000
(4) 5° C. skim 0.11% fat	54.8	200	128	48.9	40	4	46.9	275	3000
(5) 53° C. skim 0.11% fat	55.0	202	133	48.6	20	3	46.1	276	3000
(6) 53° C. skim + 53° C. cream	53.5	198	27	47.5	30	4	.....	287	3000
(7) 5° C. skim + 5° C. cream	53.5	200	30	47.4	26	4	.....	284	3000
(8) 53° C. skim + 5° C. cream	53.6	200	30	47.8	23	4	.....	286	3000
(9) 5° C. skim + 53° C. cream	53.2	197	29	47.6	26	4	.....	284	3000

Cream Volume Measurement

Sample	Cream volume %				
	Hours				
	0	2½	6½	8½	20
(2) Pasteurized milk	0	17.0	16.5	16.0	15
(6) 53° C. skim + 53° C. cream	0	15.0	14.75	14.75	14
(7) 53° 7. skim + 5° C. cream	0	15.0	15.0	14.5	14
(8) 53° C. skim + 5° C. cream	0	18.0	17.0	16.5	15
(9) 5° C. skim + 53° C. cream	0	3.5	6.5	8.0	9

fuging milk at low temperatures:[2] 2) the higher fat content in the skim milk due to inefficient separation at low temperatures.

The data on the effect of rancidity and homogenization on milk foam will be reported in a later paper. Our results show that a slight development of rancidity results in a marked decrease in the stability of milk foam at 5° and 50° C., while it increases the foam stability at temperatures between 10 and 30° C.

According to these facts, the assumption was made that if the work of Sharp and Krukovsky were to be repeated with pasteurized milk (61.5° C. for 30 minutes) and the fat were to be increased to correspond to that separated at low temperature, then both skim milks would have the same foaming properties. The assumption was borne out by the experimental results reported in table 3.

These facts do not exclude the possibility of the presence of an agglutinin in milk because the measurements obtained on the cream volume of reconstituted milks agree with the results of Sharp and Krukovsky. However, the data indicate clearly that the agglutinin is not one of the constituents which determine the foaming properties of milk.

DISCUSSION

The data presented in figure 1 show the similarity in the foaming properties of whey and synthetic solutions containing lactalbumin and lactoglobulin in their normal concentration. They show also that the addition of small amounts of milk fat to lactalbumin solutions gives foaming properties similar to whey. In other words, the reduction in the foam stability of lactalbumin solutions that results from adding lactoglobulin is probably due to the presence of small amounts of fat associated with lactoglobulin. Therefore, it is reasonable to conclude that lactalbumin and milk fat are the major constituents that determine the foaming properties of whey.

In figure 2 it is shown that the minimum foaming of separated milk at room temperature is due to the presence of fat in the separated milk. When the fat globules were largely removed by passing that separated milk through a supercentrifuge, it showed about the same foaming properties at all temperatures tested. It has been shown also that the essential foaming characteristics of supercentrifuged skim milk can be duplicated with a solution of calcium caseinate plus lactalbumin, and that the addition of 0.01 per cent milk fat to this solution decreases its foam stability to a minimum at 27° C. Similar observations on horse blood serum are reported in figure 3. These results are taken to indicate that the presence of fat globules in biological

[2] Sharp and Tomasi (21), and Doan (4) have also reported on the development of rancidity in samples separated at low temperatures and pointed out that the action of centrifugal separation is somewhat similar to homogenization in that it stimulates hydrolysis of the fat in the resulting cream.

fluids reduces their foaming properties to a great extent at certain temperatures, a fact which may prove of value in testing for lipemia.

To explain the foaming properties of skim milk or milk, we are led to the belief that calcium caseinate is the foaming substance of milk at low temperatures, while lactalbumin is the substance contributing to the foam at temperatures above the melting point of milk fat. This conclusion is supported by the experimental data presented and deduced from the following considerations.

(a) A calcium caseinate solution does not form a stable foam at temperatures above 21° C. in the presence of 0.015 per cent fat (5a). At 50° C. the "half-volume time" of such a solution is of the order of 20 seconds, while that of the ordinary skim milk or milk is over 1,000 seconds.

(b) A lactalbumin solution in the presence of 0.005 per cent fat does not form a stable foam at temperatures below 34° C. but forms a stable foam at higher temperatures corresponding to those of milk and of skim milk (5a).

TABLE 4

No. of times through separator	Sample	Protein			
		Total	Casein	Heat coagulable	Non-heat coagulable
		%	%	%	%
2	Original milk	3.89	3.05	0.40	0.44
	Foam	4.19	3.35	0.40	0.44
	Difference	0.30	0.30	0.0	0.0
5	Original milk	3.69	2.89	0.37	0.43
	Foam	4.00	3.20	0.35	0.45
	Difference	0.31	0.31	−0.02	+0.02
8	Original milk	3.57	2.77	0.37	0.43
	Foam	3.82	3.00	0.39	0.43
	Difference	0.25	0.23	0.02	0.0

(c) On mixing solutions of calcium caseinate and lactalbumin with or without the addition of fat, the essential characteristics of the foaming properties of the corresponding separated milk can be duplicated (figure 2). On the other hand, the addition of lactoglobulin to a calcium caseinate solution does not alter the foaming properties of the latter to any great extent (table 1).

(d) At temperatures below 30° C., skim milk gives a more stable foam than the acid or rennet wheys separated from it. This is due to the absence of calcium caseinate from the whey.

The conclusion is contrary to the prevailing ideas in the dairy field, but it is further justified by the data of other workers. Sharp, Myers and Guthrie (20) investigated the accumulation of protein in the foam of skim milk and came to the conclusion that "there is no preferential accumulation

of any major protein fraction in the foam." Table 4, compiled from their data, indicates that there is a preferential adsorption of casein in the foam at 10° C. which is in complete agreement with the present conclusion.

The preferential adsorption of the substance having the highest surface activity at the air solution interface is in accord with thermodynamical principles and has been reported by many investigators (2, 6, 7, 8, 16, 23, 24). Other workers have found this principle useful in biochemical studies (13, 14).

Ansbacher, Flanigan and Supplee (1) eluted commercial casein with sodium chloride and claimed that they obtained the foaming substances from casein, since the eluted casein did not foam appreciably. They did not, however, present data on this foaming substance or of the casein before and after elution. In a previous paper (5a) we showed that when casein is eluted with 4 per cent NaCl at pH 4.2, the foaming properties of the calcium caseinate prepared were not reduced. Considering that casein at its isoelectric point is soluble in 0.115 N NaCl solutions to the extent of 3.46 gm. per liter (17) and that casein has its maximum foaming capacity at a concentration of about 0.05 per cent, the complications in the studies of these workers may be realized.

Another attempt to assign the foaming properties of separated milk to a minor fraction of its proteins has been reported recently by Sharp and Krukovsky (19). These workers reported two paradoxical ideas; while they did not think that less efficient separation and consequently higher fat content in the skim milk separated at low temperature was the cause of its poor foaming properties, they reported that when this separated milk was extracted with petroleum ether its foaming properties then approached those of the high temperature separated milk. This result would seem to indicate the presence of an anti-foamer (lipoid) prior to extraction but not the absence of an agglutinin. Our results show the "agglutinin" is not important in the foaming of separated milk.

SUMMARY

Synthetic solutions of milk proteins and fat have been prepared which duplicate the essential foaming characteristics of whey and skim milk. Lactalbumin and milk fat were found to be the constituents that influence the foaming of whey. The constituents in milk or skim milk that determine its foaming properties are shown to be calcium caseinate, milk fat and lactalbumin. It is considered that calcium caseinate is preferentially adsorbed at the air/liquid interface at temperatures below the melting point of milk fat, lactalbumin being adsorbed at higher temperatures. The presence of milk fat globules is shown to be responsible for the minimum foaming of separated milk at certain temperatures. The addition of milk fat emulsion to blood serum brings about a minimum foaming at room temperatures.

Previous work which is not in agreement with the present conclusion is analyzed in the light of the present studies.

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A STUDY OF METHODS OF OBTAINING MILK SAMPLES FOR ESTIMATING MILK FAT BY THE MOJONNIER METHOD

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Wide differences in the amounts of milk fat were obtained from some samples of the same milk by the Babcock and by the Mojonnier methods. An examination of the data revealed that the method of weighing the samples of milk and of transferring them into the extraction flask by the Mojonnier method was responsible for these abnormal variations.

Though not recognized as official, the Mojonnier method is used extensively in dairy laboratories in this country instead of the Röese-Gottlieb method (1). Some essential details of the technic for obtaining the sample of milk are lacking in the Röese-Gottlieb method (1) and three different technics are used in the Mojonnier procedure (3, 4).

The writers were unable to find any data in the literature on this subject, except a statement by Dahlberg, Holm and Troy (2) which is quoted. "It was thought that the measuring of samples would introduce slight errors and that fat might rise in the weighing pipette during the time required for weighing to such an extent that extra rich milk might remain in the pipette after the sample was delivered. Both practices were discontinued. After the samples had been heated and mixed until they appeared homogeneous, the milk required for one analysis was quickly poured out and weighed. The samples were weighed directly into the Mojonnier tubes at one laboratory, and at the other, they were weighed into small 25-cc. flasks and transferred to Röhrig tubes, using all of the chemicals used in the test to rinse out the flasks." Therefore it was deemed advisable to compare the different methods of transferring and of weighing samples of milk into Mojonnier extraction flasks.

PROCEDURE

The milk samples were heated to 35 to 37.5° C. in a water bath at 41.5 to 43° C., and mixed by pouring three times from one container to another. The pipettes in all three methods were filled so that the bottom of the menisci were level with the mark on the draw tubes. Each pipette delivered slightly less than 10 grams of milk at 35–37.5° C.

In method one, four pipettes were filled, the milk sample being poured once into another container between the filling of each pipette. As each

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[2] Published with the approval of the Director of the Vermont Agricultural Experiment Station.

pipette was filled, it was placed on a holder and the holder with the filled pipettes was placed on the balance pan. When the total weight was obtained, pipette number 1 was emptied into extraction flask number 1 and allowed to drain for 10 to 15 seconds. This empty pipette was again placed on the holder and the weight of milk delivered into the flask was obtained by difference. This procedure was repeated to obtain the weight of milk delivered by pipettes number 2, 3 and 4 into each of three extraction flasks that were similarly numbered. The weight of the four pipettes full of milk and the weighing stand is about 112.5 grams.

In method two, a charge of milk, using pipette number 1, was obtained from the sample, transferred immediately to a tared extraction flask, allowed to drain for 10 to 15 seconds after the flow had ceased and weighed. This was done in duplicate on each sample. The weight of the flask and charge of milk is about 76.1 grams.

In method three, a charge from the milk sample, with pipette number 1, was transferred volumetrically to an extraction flask and allowed to drain for 10 to 15 seconds after the flow had ceased. This was done in duplicate on each sample. In recording the results it is assumed that the pipette delivered 10 grams of milk.

Composite milk samples preserved with bichloride of mercury were obtained from two nearby milk plants and were sampled and transferred by methods one and two and estimated for milk fat. These plants are designated A and B. Plant A had excellent facilities for storing the composites during the bi-monthly period. They were kept constantly in a refrigerated compartment at 10° C. or lower. At the end of the bi-monthly period the samples were free of mold growth and in excellent physical condition. Plant B did not have such good facilities. The preserved milk composites were heavily contaminated with mold at the end of the bi-monthly period and the fat emulsion showed varying degrees of destabilization when tested at the milk plant. These composites were heated and prepared for testing the same as the unpreserved samples except that the cream adhering to the inside of the sample bottle was brushed loose. These preserved samples had been prepared and estimated once for milk fat by the Babcock method in the milk plant before they were brought to the Experiment Station laboratory. All the samples were selected, insofar as possible, to represent high, medium and low fat content milks in each trial.

The Mojonnier (3, 4) gravimetric procedure, equipment and prescribed reagents were used to estimate the milk fat. This is a mechanized modification of the Röese-Gottlieb method. Blank determinations were made on new supplies of reagents. The ether extractions on replicate samples obtained by the three methods were made at the same time. The temperature of the laboratory and equipment did not vary greatly from 21° C., the importance of temperature having been emphasized by Mojonnier (3) and Troy (6).

RESULTS

The results in table 1 show the effect of three methods of weighing and transferring 30 unpreserved and 120 preserved milk samples into extraction flasks. When milk samples are weighed in pipettes on the weighing stand (method one), there is a gradual decrease in the amount of milk fat from

TABLE 1

The effect of different methods of transferring preserved and unpreserved milk samples into extraction flasks on the estimation of milk fat by the Mojonnier method

Number of samples	Mean per cent milk fat							
	Weighed in						Measured in	
	Pipettes—Method one				Flasks—Method two		Flasks—Method three	
	1	2	3	4				
Unpreserved								
10	4.6126	4.5746	4.5260	4.5156	4.6222	4.6097	4.5849	4.5800
10	3.6572	3.6386	3.5859	3.5891	3.6949	3.6723	3.6449	3.6604
10	3.3385	3.3129	3.2897	3.2900	3.3550	3.3411	3.3225	3.3134
Preserved								
Plant A								
First Trial								
10	4.2701	4.2293	4.1541	4.1311	4.2991	4.3061		
10	3.5434	3.5395	3.4976	3.4539	3.5693	3.5680		
10	3.2142	3.1927	3.1491	3.1423	3.2332	3.2363		
Second Trial								
10	4.2718	4.2490	4.1845	4.1625	4.3031	4.2934		
10	3.5710	3.5564	3.5135	3.4994	3.6061	3.6076		
10	3.1420	3.1311	3.0821	3.0804	3.1731	3.1782		
Plant B								
First Trial								
10	4.5192	4.4844	4.3808	4.3876	4.6055	4.5980		
10	3.8707	3.8611	3.7870	3.7568	3.9230	3.9130		
10	3.4147	3.4008	3.3496	3.3371	3.4533	3.4482		
Second Trial								
10	4.7224	4.7059	4.6517	4.6315	4.7990	4.8054		
10	3.8834	3.8814	3.7996	3.7874	3.9421	3.9413		
10	3.5008	3.5010	3.4392	3.4348	3.5499	3.5538		

pipettes 1 to 4. The results from pipette number 1 (method one) agreed closely, but averaged slightly lower than those where the samples were weighed directly into the extraction flasks (method two). Measuring milk by volume and assuming that the pipette delivered 10 grams of milk, yielded lower results than weighing it into extraction flasks. Pipette number 1

delivered an average of 9.9402, 9.9200 and 9.9314 grams of the high, medium and low fat unpreserved milks, respectively, at 35–37.5° C. When calculations are made for the weight of the unpreserved milk delivered by this pipette, then the results obtained by measuring and by weighing milk into extraction flasks agree closely.

The results obtained by weighing the samples from Plant A into extraction flasks (method two) are slightly higher than those from pipette number 1 (method one). However, the same comparison from Plant B indicates

TABLE 2

A distribution of results from the pipettes that yielded the highest estimation of milk fat by method one on replicate samples

Milk source	Pipette number			
	1	2	3	4
Unpreserved	9	1	.....	.....
	9	1	.....	.....
	8	.....	.....	2
Preserved				
Plant A				
First trial	8	2	.....	.....
	4	5	1	.....
	9	1	.....	.....
Second trial	9	1	.....	.....
	9	1	.....	.....
	10	.....	.....	.....
Plant B				
First trial	9	1	.....	.....
	6	4	.....	.....
	8	2	.....	.....
Second trial	8	2	.....	.....
	6	4	.....	.....
	6	2	1	1
Total	118	27	2	3
Per cent of total	78.67	18.00	1.33	2.0

that significantly higher results were obtained by method two. This can be attributed to the destabilized condition of the fat emulsion in the preserved milk composites from Plant B resulting in more rapid rising of the destabilized fat in the pipettes.

The distribution of results in table 2 indicates that of the 150 estimations for milk fat that were made in replicate samples with method one, pipette number 1 yielded the highest results on 118 samples, pipette number 2 on 27 samples, pipette number 3 on 2 samples and pipette number 4 on 3 samples. The percentage distribution is 78.67, 18.00, 1.33 and 2 per cent for pipettes number 1, 2, 3 and 4, respectively. Pipette number 2 yielded the highest results on 10 unpreserved samples from Plant A and on 15 unpre-

served samples from Plant B. It is evident that method one is not an accurate procedure to use in weighing and transferring milk samples to extraction flasks.

DISCUSSION

This study has shown that method one is not an accurate procedure for weighing and transferring samples of milk to the extraction flasks. This method is not reliable because the clusters of fat rise according to Stoke's Law and are adsorbed to the inner surface of the pipettes. The amount of cream thus adsorbed depends on the length of time that the pipettes are on the weighing stand. The amount is least in pipette number 1 and greatest in number 4. The inside diameter of the pipettes is slightly less than two centimeters. The time required to weigh and transfer four pipettes of milk (method one) to the four extraction flasks was 10.3 minutes for one technician and 12.6 minutes for the other. This would be sufficient time for some of the clusters of cream to rise in the pipettes according to the calculations by Sommer (7). In spite of the fact that four charges can be obtained with five weighings, method one is not reliable for whole milk when a high degree of accuracy is desired. The error is not great for pipettes 1 and 2, but is significant for pipettes 3 and 4. Method one was developed by Mojonnier Brothers (3, 4) especially for evaporated milk, sweetened condensed milk, ice cream, and other milk products of a homogeneous nature, but technicians might interpret the instructions to apply this method to the estimation of milk fat in whole milk. The senior author has observed the use of method one on milk in several laboratories. This method saves time because four samples can be obtained with only five weighings, while eight weighings must be made when the samples are transferred directly into the extraction flasks. The errors involved in method one would not have been considered serious twenty years ago, but now are significant with the trend toward greater accuracy and efficiency in the dairy industry. Mojonnier Brothers (5) recently emphasized that the weighing stands with pipettes are intended to be used only for products in which the fat does not readily separate.

Method two is the most reliable. It is possible to obtain a charge of milk from a well-mixed sample and deliver it immediately to the extraction flask. This is especially important in sampling milks where the fat emulsion is partially destabilized, because the destabilized fat rises rapidly in the sample jar as well as in the pipette. Therefore, it is highly important to obtain the sample quickly and deliver it immediately into the extraction flask. Mojonnier (3, 4) recommends this method for products that are not homogeneous or when the milk fat separates rapidly. When method two was used, duplicate determinations for milk fat agreed closely.

Method three may be used in commercial laboratories, provided the pipette is calibrated to deliver a definite charge of milk under standard conditions. The volumetric procedure is recommended by Mojonnier Brothers

(3, 4) but they are careful to specify that their pipettes are calibrated to deliver 10 grams of milk at 15.5° C., allowing 15 seconds for the pipette to drain after the milk has ceased to flow and blowing out the last drop. A sampling temperature of 35–37.5° C., was used in this study, because it was assumed that a more representative sample would be obtained and that the pipettes would drain more completely when the milk fat is liquid. This higher sampling temperature is at least one reason why the pipettes did not deliver the specified 10 grams of milk.

CONCLUSIONS

1. Weighing milk in four pipettes on the weighing stand and transferring it to extraction flasks is not an accurate procedure. (Method one.)

2. Weighing milk directly into a tared extraction flask is the most reliable of the three methods. (Method two.)

3. Measuring milk by volume into the extraction flask with a pipette will give sufficiently accurate results for routine work provided the pipette is calibrated for the amount of milk that it will deliver under standardized conditions. (Method three.)

4. For sampling and delivering the milk sample into the extraction flask with a pipette in methods two and three, the thoroughly mixed milk should be at a temperature slightly above the melting point of the fat; for example 35–37° C.

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CHLORINE RESISTANCE OF *PSEUDOMONAS PUTREFACIENS**

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In the attempts to control outbreaks of the putrid defect in butter, chlorine solutions frequently are used to treat butter wash water and sometimes to treat plant equipment, especially churns. The difficulty in controlling the outbreaks with such procedures has suggested to certain plant operators that the causative organisms are relatively resistant to chlorine, although there remains the possibility that the suspected source of the organisms—wash water or plant equipment—was not the actual source so that the organisms were not exposed to the chlorine.

Since *Pseudomonas putrefaciens* is a common cause of the putrid defect in butter (1, 3), its chlorine resistance was investigated. Some of the cultures used were stock cultures, while others were freshly isolated for the study. The problem of chlorine resistance of *Ps. putrefaciens* is greatly complicated by the difficulty in growing the organism (2). In the studies an attempt was made to find the conditions under which it grows best.

The usual procedure was to suspend a 2-day agar culture of *Ps. putrefaciens* in sterile or pasteurized water, allow the suspension to stand for a short time at room temperature and then treat portions with the required amounts of a hypochlorite solution containing 100 ppm. chlorine. After the desired exposures, action of the chlorine was stopped by adding a solution of sodium thiosulfate. Occasionally, two concentrations of chlorine were used on the same original suspension. With each type of water some of the trials included titration of the chlorine at the beginning and end of the exposures; commonly, the chlorine concentration did not decrease significantly during an exposure and the values given represent concentrations at the ends of the exposures. The untreated and treated suspensions were cultured on special agar (2) and in some instances were used to wash butter granules obtained by churning thoroughly pasteurized sweet cream in sterile glass jars; in a few trials they also were cultured in milk.

RESULTS

Trials with sterile distilled water. Addition of 1 ppm. chlorine to a suspension of *Ps. putrefaciens* in sterile distilled water usually resulted in death of the organism in 5 seconds, but occasionally survival was noted for as long as 3 minutes; generally, suspensions requiring longer treatment contained more organisms originally than those requiring shorter treatment although other factors may have been involved. In 10 minutes the organism regularly was killed. With 5 ppm. chlorine the organism never survived a

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TABLE 1

Action of chlorine on Ps. putrefaciens suspended in well water

Trial	Strain of organism	Material examined	*Ps. putrefaciens* per ml.*	Butter washed with material examined†
1	1	Inoc. well water	40,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 2 min.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 5 min.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 10 min.	< 1	No change in 4 days
2	1	Inoc. well water	30,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 10 sec.	< 1	Slightly off in 4 days‡
		Inoc. well water + 5 ppm. Cl for 30 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 2 min.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 5 min.	< 1	No change in 4 days
3	1	Inoc. well water	54,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 5 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 10 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 30 sec.	< 1	No change in 4 days
		Inoc. well water + 5 ppm. Cl for 2 min.	< 1	No change in 4 days
4	2	Inoc. well water	12,000	Putrid in 2 days
		Inoc. well water + 5 ppm. Cl for 5 sec.	< 1	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 30 sec.	840	Putrid in 2 days
		Inoc. well water + 1 ppm. Cl for 2 min.	< 1	No change in 4 days
		Inoc. well water + 1 ppm. Cl for 10 min.	< 1	No change in 4 days
5	3	Inoc. well water	16,000	
		Inoc. well water + 1 ppm. Cl for 1 min.	Plates overgrown	
		Inoc. well water + 1 ppm. Cl for 5 min.	Plates overgrown	
		Inoc. well water + 1 ppm. Cl for 10 min.	130	
		Inoc. well water + 1 ppm. Cl for 20 min.	< 1	

* As determined by smears on special agar.
† Unsalted butter held at 21° C.
‡ Tests indicated that *Ps. putrefaciens* was not present.

5-second exposure, which was the shortest one used. During trials with this type of water it was noted that without chlorination *Ps. putrefaciens* remained viable for only a few hours. While survival for 1 hour, or even much less, was sufficient for conducting the tests on a suspension, it appeared that killing the organism with chlorine in this type of water would be easier than in the type of water generally used in dairy plants. Accordingly, additional tests were conducted.

Trials with pasteurized well water. Water was obtained from the college water system before filtration; after pasteurization at approximately 90° C. for 30 minutes, it was cooled to room temperature and filtered to remove the precipitated material. Table 1 gives representative results obtained with this type of water.

In trial 1 with strain 1 the original suspension of organisms had a count of 40,000 per ml. and 5 ppm. chlorine killed them with exposures of 2, 5 or 10 minutes. The unchlorinated water produced a putrid condition in unsalted butter in 2 days at 21° C. while the chlorinated samples failed to produce the defect. In trials 2 and 3, also with strain 1, satisfactory destruction of the organisms was obtained with 5 ppm. chlorine, even with very short exposures. With an exposure of only 5 seconds (trial 3) no growth was obtained on plates and no spoilage (due to *Ps. putrefaciens*) resulted when the treated water was used to wash butter. The effect of a smaller dosage of chlorine is shown in trial 4 conducted on a suspension of strain 2 containing 12,000 organisms per ml. Although 5 ppm. chlorine for 5 seconds resulted in satisfactory destruction, 1 ppm. for 30 seconds failed to kill all the organisms and unsalted butter washed with the water became putrid in 2 days at 21° C.; however, exposures of 2 or 10 minutes resulted in satisfactory destruction. In trial 5, conducted on a suspension of strain 3 containing 16,000 organisms per ml., 1 ppm. chlorine failed to give as satisfactory destruction as in trial 4 and there was some survival after 10 minutes exposure although all the organisms were killed in 20 minutes; these suspensions were not tested in experimental churnings. Presumably strain 3 was comparatively resistant to chlorine.

The data indicate that *Ps. putrefaciens* is rather susceptible to destruction by chlorine, being rapidly killed, even when present in comparatively large numbers, with a concentration of 5 ppm. With less chlorine more time is required, but again the organism displays no unusual powers of resistance. Under certain conditions, such as presence of organic matter in the water, excessive numbers of organisms or some comparable factor, less satisfactory destruction would be expected. The type of chlorine compound and the pH at which it acts need definite consideration.

Trials involving unsatisfactory destruction of Ps. putrefaciens. In certain trials chlorine failed to give satisfactory destruction of *Ps. putrefaciens*, even when allowed to act for considerable periods; the following examples illustrate this.

Example 1. A suspension of 480,000 organisms per ml. in lake water (pasteurized and filtered) was treated with sufficient chlorine to give a residual of 0.9 ppm. after 2 minutes. Under these conditions the chlorine failed to kill enough organisms in 5 seconds, 10 seconds, 1 minute or 2 minutes to show any destruction on agar plates smeared with 1 ml. or 0.1 ml. of the treated suspensions. In addition, all the chlorinated samples produced spoilage in experimental butter in the same period as the unchlorinated suspension.

Example 2. Well water (pasteurized and filtered) with a count of 620,000 organisms per ml. was treated with 1 ppm. chlorine for periods up to 2 minutes. All the exposures used failed to give appreciable decreases in the number of organisms. In another trial with well water containing 60,000 organisms per ml., 2 ppm. chlorine failed to give satisfactory destruction even after 10 minutes.

Presumably, various factors are involved in the low destruction of *Ps. putrefaciens* in certain trials. In example 1 the number of organisms was excessive for the amount of chlorine and the periods of action; in addition, the water evidently contained considerable organic matter since chlorine was rapidly dissipated in it without any addition of organisms. In the first trial of example 2 an excessive number of organisms in relation to the amount of chlorine and periods of action was again involved. However, in the second trial this apparently was not the case although the strain employed was a very recent isolation which may have grown poorly on the agar so that the count obtained was much lower than the actual number of cells present; apparently, the strain was not especially resistant because in later trials it was easily destroyed by chlorine.

Growth of Ps. putrefaciens in the test media employed. When the untreated and treated suspensions of *Ps. putrefaciens* were cultured on the surface of the special agar, growth usually was satisfactory but in a few instances suspensions failed to give the growth expected on the basis of the amount of inoculum used. This was particularly true with certain strains of *Ps. putrefaciens* and especially with some very recent isolations.

Comparisons of growth of *Ps. putrefaciens* on the special agar and in litmus milk indicated that the agar was the better medium. Often suspensions giving good growth on the agar failed to produce any change in litmus milk, even with comparatively large inoculations. There was an excellent agreement between growth of the organism on the agar and production of the putrid defect in unsalted butter at 21° C. Even when a suspension gave comparatively little growth on plates it eventually caused the defect in butter. Commonly, results were obtained more quickly on the agar, where colonies usually were evident in 24 to 30 hours, than in butter; in most cases 2 days or longer were required for production of the putrid defect in butter, even when the wash water contained many organisms.

On the basis of the results it appears that culturing on the special agar is a satisfactory method of measuring destruction of *Ps. putrefaciens* although even on this medium some cells apparently fail to grow. The agreement between growth on the agar and production of the putrid defect in butter is significant from the practical standpoint since satisfactory destruction of the organism has been attained when it does not produce a defect in butter.

DISCUSSION

Failure of *Ps. putrefaciens* to show unusual chlorine resistance would be expected because of the absence of spores and the general lack of resistance of the species of the genus *Pseudomonas* to various agents causing destruction of bacteria. When unsatisfactory destruction occurred, the cause generally was apparent and involved factors other than resistance of the organism to chlorine.

The excellent agreement between growth of *Ps. putrefaciens* on the special agar and in butter is rather surprising since it often is assumed that butter is the best medium for the organism. The comparatively poor growth in litmus milk has been noted in other investigations (4).

SUMMARY

When suspended in sterile distilled or pasteurized and filtered well water, *Ps. putrefaciens* was easily destroyed by chlorine, provided excessive numbers of cells were not present; destruction was especially active in the distilled water. When the numbers of organisms in relation to the amount of chlorine or its period of action were excessive, destruction was unsatisfactory. Among the strains tested there was some evidence of variation in chlorine resistance.

There was excellent agreement between growth of *Ps. putrefaciens* on a special agar and production of the putrid defect in unsalted butter at 21° C. Various suspensions yielding good growth on the agar failed to produce changes in litmus milk.

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OBSERVATIONS ON FISHINESS IN BUTTER*

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At one time fishiness in butter was a common and conspicuous defect. Regardless of the original quality, butter rather frequently developed a pronounced fishy flavor, especially during storage, and the financial losses involved were large. Because of its importance, the defect has been extensively investigated and the more significant factors favoring its development are now generally recognized (2, 3, 4, 5).

While at present fishiness in butter is being controlled to a large extent, the defect still is occasionally encountered. Often this fishiness is rather mild and the pronounced fishy flavors that at one time caused such serious financial losses are unusual. The mild fishiness is not generally recognized as such by the consumer, and the criticism may simply be that the butter is off in flavor.

For the most part the fishiness now encountered in butter is due to failure to apply the control procedures that have been thoroughly established. Illustrations of such failures are reported herein; all of them involve salted butter, fishiness being rare in unsalted butter.

RESULTS

Fishiness with low pH values. In all probability reduction of the acidities at which cream is churned has been the most important factor in limiting the number of fishy churnings and the intensity of the fishiness in those churnings. However, it appears that insufficient acid reduction still is of significance as a cause of the defect.

Table 1 gives the pH values of fishy and non-fishy butter from various plants; the churnings in each series were made within a very short period so that the general conditions of manufacture were much the same, but since each plant obtained cream from a relatively large area there undoubtedly were definite differences in the cream used. In each series the churning or churnings of butter having a fishy flavor had the lowest pH value or values. In some series the difference in pH between a fishy and a non-fishy churning was small.

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TABLE 1

The pH values of fishy and non-fishy lots of commercial butter from various plants
The 2 to 6 samples from a plant were made during a short period.

Plant no.	Sample no.	Fishy flavor	pH	Plant no.	Sample no.	Fishy flavor	pH
1	1	–	6.75	5	1	–	6.76
	2	+	6.05		2	+	6.37
	3	–	6.56		3	–	6.93
	4	–	6.80		4	+	6.22
					5	+	6.46
2	1	+	6.45		6	–	7.24
	2	+	6.58				
	3	–	7.04	6	1	–	6.97
	4	–	6.82		2	+	6.51
					3	–	6.91
3	1	–	7.32		4	+	6.47
	2	+	6.58				
	3	–	7.64	7	1	–	7.02
	4	–	7.08		2	+	6.55
					3	–	6.67
4	1	–	6.96				
	2	–	6.78	8	1	+	6.68
	3	+	6.52		2	–	7.02
	4	+	6.64				

Distribution of the pH values of a number of fishy and non-fishy churnings of butter from each of four plants is shown in figure 1. The churnings from each plant were not necessarily consecutive but were made within a comparatively short period. In general, the fishy churnings from a plant are grouped among the churnings having relatively low pH values. Also,

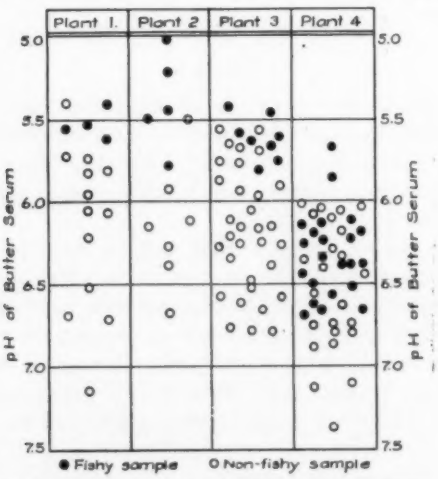

FIG. 1. Distribution of pH values of fishy and non-fishy samples of butter from four plants.

the butter from plant 4 commonly developed fishiness at higher pH values than the butter from the other three plants, suggesting that a factor other than pH also was operative.

The pH values of miscellaneous samples of fishy butter from widely scattered sources are given in table 2. In general, the values are lower than those desired in butter by various manufacturers who attempt to control the pH of their butter within rather narrow limits because of the effect of low pH on chemical deterioration in butter. Some of the values are so low that fishiness definitely would be expected to develop in the butter.

Fishiness with relatively high pH values. Occasionally lots of fishy butter have pH values which ordinarily would be considered high enough to prevent development of the defect since they definitely are above the values commonly associated with fishy butter. Because of the relationship of copper to chemical deterioration in butter, a number of such samples were

TABLE 2

The pH values of miscellaneous samples of fishy butter from widely scattered sources

Sample no.	pH	Sample no.	pH
1	5.61	11	5.35
2	5.98	12	5.95
3	5.75	13	6.15
4	5.45	14	6.14
5	6.50	15	5.98
6	6.74	16	5.88
7	6.63	17	6.44
8	6.51	18	5.68
9	6.60	19	5.35
10	5.10	20	5.80

examined for copper content, using wet ashing (6) and the carbamate procedure (1, 6). High values commonly were obtained.

The general situation is illustrated by the following case history: In a plant receiving cream from a wide area, special attention was being given to the control of pH in the finished butter, and for the most part variations in pH values of the different churnings were small. At one of the routine examinations of samples from successive churnings in the plant, the flavor scores were normal except in the case of one sample which was decidedly fishy; the samples had been held for about 3 weeks at about 7° C. so that conditions were favorable for development of the defect. It was assumed that the pH of the sample was low, but determinations indicated a value of 7.1. Copper determinations were then made and showed a content of 7.3 ppm. Other churnings made on the same day as the fishy butter contained from 0.4 to 0.75 ppm. of copper.

The plant records for the day on which the defective butter was made showed that the vat of cream used for the butter had a conspicuously lower

acidity than any of the other vats. It included a large proportion of cream from cheese factories, both that from whey and that from milk; this type of cream was reaching the plant at lower acidities than cream coming from farms, either directly or through cream stations. In various cheese factories exposed copper on equipment undoubtedly accounted for a high copper content in the whey and in the cream obtained from it.

Table 3 gives copper contents of additional samples of fishy butter having pH values so high that fishiness ordinarily would not be expected in the butter. The contents vary from 0.85 to 7.0 ppm. While data on the normal copper contents of butter in the areas involved are not available, the values given are definitely higher than those on other churnings from the same plants at approximately the same periods.

TABLE 3

Copper contents of samples of fishy butter having relatively high pH values

Sample no.	pH	Cu content
		ppm.
1	6.70	1.3
2	6.70	1.9
3	7.19	1.3
4	7.25	7.0
5	6.85	2.2
6	7.10	1.9
7	7.13	0.85

In a number of instances fishy butter was encountered which had both a high copper content and a relatively low pH value. Under such conditions the effect of the copper probably was greater than it would have been with a high pH value.

Control of the pH of butter. Adequate control of the pH of salted butter, which is so necessary under present conditions of butter manufacture, sometimes presents difficulties. Proper adjustment of the acidity of the cream at the time of churning is a common basis for such control. Under a given set of plant conditions there is a reasonably close correlation between cream acidity at churning and pH of the butter but, because of the influence of various factors on the relationship, results obtained in one plant must be tested in another plant before they can be accepted there. Also, in a plant in which the correlation has been thoroughly investigated an unusual condition may upset the relationship which has prevailed for an extended period.

In a plant which began to receive considerable volumes of cheese factory cream, both whey cream and cream from whole milk, it was noted that the butter was developing a fishy flavor soon after manufacture. Study of the butter showed that the pH values were relatively low although the churning acidities had not been changed. Eventually it was learned that certain

cheese factories were skimming a very rich cream and then diluting it with cold water to cool the cream and thin it sufficiently to make handling easier. Addition of the water changed the buffer capacity of the cream so that the pH of the cream at a given acidity was considerably lower than it would otherwise have been.

Sources of copper in butter. Equipment in certain manufacturing plants is an obvious source of copper in butter. Exposed copper in various vats, in piping, etc., contributes significant amounts of copper to cream and to the butter made from it. Presumably an alloy containing copper also can be of importance in this connection. The definite trend toward the use of special metals in the making of equipment for various types of dairy plants is a clear recognition of the objectionable effects of copper and certain other metals on dairy products.

There also are less obvious sources of copper in cream and butter, such as exposed copper in cheese plants supplying cream, unusual equipment on dairy farms, etc. Some of these may be difficult to detect because of their distance from the butter plant but should be considered when it appears that such defects as fishiness in butter are caused by excessive amounts of copper in the product.

When replacement of equipment showing exposed copper is impossible for financial or other reasons, retinning limits the copper contamination. Also, butter made from the first cream through the equipment each day should be used in channels in which it will soon be consumed rather than in channels in which consumption may be delayed. It is probable that there is a definite advantage in keeping the pH of the butter comparatively high.

Detailed studies on the normal copper content of butter are greatly needed so that it will be possible to tell when the content has been definitely increased during the collection of cream and its manufacture into butter. There is some evidence that the normal copper content of butter from various areas differs rather widely.

Distribution of fishiness between fat and serum of butter. In a number of instances the distribution of fishy flavor between fat and serum of butter was studied by melting the butter and separating the fat and the serum with a separatory funnel. The melted fat was then filtered through paper at about 45° C., and the serum was centrifuged to remove the fat as completely as possible.

The fishy flavor was very conspicuous in the fat, while there was little or no fishiness in the serum. When experienced butter judges were asked to taste the serum and identify the defect in the butter from which the serum came, they commonly could not do so; however, if the fat was not rather completely removed from the serum the fishiness was evident in the serum.

When the filtered butterfat was steam distilled the distillate was very fishy, but even after extended distillation some fishiness was still evident in

the steamed fat. In general, the steaming had no significant effect on the acidity of the fat.

DISCUSSION

The data emphasize the importance of proper pH control in the manufacture of butter if fishiness is to be avoided and are in agreement with the fundamental studies that have been reported along this general line. They also show that fishiness still occurs in butter from various plants. Proper control of the pH of butter apparently is not as simple as it sometimes is supposed to be. This is particularly true in plants receiving cream from a variety of sources, some of which may present unusual situations from the standpoint of the relationship between the titration value on the cream and the pH of the resulting butter.

The presence of relatively large amounts of copper in butter developing fishiness when the pH values are satisfactory again is in agreement with the results of the early investigations. While contamination of cream and butter with copper in butter manufacturing plants can be controlled under normal conditions by replacement of equipment, retinning of equipment, etc., it is probable that with plants receiving cream from certain sources the cream sometimes contains excessive amounts of copper when it reaches the plants. This general situation shows the importance of a proper understanding of the conditions under which cream is handled before it comes to the butter plants.

Evidence from various sources indicates that factors other than pH and copper content influence the development of fishiness in butter. In addition to those commonly recognized, the area in which the cream is produced and the cream treatment appear to be of importance on the basis of general observations.

The cream from certain producing areas apparently yields butter which is more susceptible to the development of a fishy flavor than cream from other areas. This could easily be related to the feeds consumed by the cows because of their effect on the composition of the milk, including the content of such minor constituents as lecithin (5). However, there also are other possibilities.

Extensive treatment during the processing of cream for butter also may influence the development of fishiness. For example, certain types of pasteurization may affect the stability of the fat or fat-like constituents.

SUMMARY

Fishy butter from different plants commonly had lower pH values than non-fishy butter from the same plants at about the same periods. In various instances in which fishy butter had a relatively high pH value, it contained comparatively large amounts of copper.

Proper control of the pH of butter requires recognition of any unusual condition which develops. Certain sources of copper are very obvious but others, such as exposed copper in cheese plants supplying cream for butter manufacture, are much less obvious.

When fishy butter was separated into fat and serum, the fishy flavor was conspicuous in the fat but there was little or no fishiness in the serum.

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FINAL REPORT OF THE SUB-COMMITTEE ON THE DETERMINATION OF THE PERCENTAGE OF FAT IN SWEETENED CONDENSED MILK AND EVAPORATED MILK

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The committee has made an attempt to find a satisfactory method for the determination of fat in sweetened condensed milk and evaporated milk where Mojonnier equipment is not available.

The standard method of procedure in conducting the Babcock test is unsuitable for testing dairy products containing added sugar on account of the carbonization of the sugar which makes the reading of the fat column difficult.

Accuracy, simplicity, low cost and the use of standard Babcock equipment were the principal factors considered.

Comparisons were made of a number of methods which have been used in determining the percentage of fat in dairy products, with the Mojonnier method as the standard. Some of the methods investigated were eliminated because the originators did not recommend them for testing sweetened condensed milk and evaporated milk. Others were eliminated because the results were not satisfactory to the committee.

It was found, in making a further investigation, that both the Pennsylvania and the Minnesota methods gave results which agreed closely with the Mojonnier method. It was decided to recommend both of these methods.

PREPARATION AND AMOUNT OF SAMPLE

I. *Sweetened Condensed Milk*

To facilitate weighing the sweetened condensed milk into the test bottle, it can be mixed with an equal weight of water. This is done by balancing two beakers on opposite sides of a set of cream or similar scales; approximately 4 ounces of sweetened condensed milk are added to one of the beakers and an equal weight of water added to the other and the contents of the two beakers are thoroughly mixed together and then placed in a stoppered flask.

For both the Pennsylvania and Minnesota methods, weigh 9 grams of this mixture into either a 9-gram, 20 per cent ice cream test bottle or an 8 per

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cent milk test bottle. The results are multiplied by two if a 9-gram ice cream test bottle is used and by four if an 8 per cent milk test bottle is used.

II. *Evaporated Milk*

In the Pennsylvania method, weigh a 6-gram sample into either an 8 per cent milk test bottle or a 9-gram ice cream test bottle. Multiply the results by three when an 8 per cent milk test bottle is used and by one and one-half when a 9-gram ice cream test bottle is used.

For the Minnesota method, weigh a 9-gram sample into a 9-gram 20 per cent ice cream test bottle.

DIRECTIONS FOR USING THE PENNSYLVANIA METHOD

I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

II. *Reagents*

1. Ammonium hydroxide (28–29 per cent NH_3 is necessary).
2. Normal butyl alcohol (B.P. 117° C.).
3. Diluted commercial sulphuric acid (specific gravity approximately 1.72–1.74). The acid is diluted by adding 3½ parts, by volume, of commercial sulphuric acid, specific gravity 1.82–1.83, to one part of water, in a heat resisting container.

III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk even though the weight of sample varies. The procedure is as follows:

1. Add 2 milliliters of ammonium hydroxide from a burette.
2. Mix for approximately one-half minute.
3. Add 3 milliliters of butyl alcohol from a burette.
4. Mix for approximately one minute.
5. Add 17.5 milliliters of the diluted sulphuric acid.
6. Mix thoroughly until digestion is completed.
7. Centrifuge the bottles for 5 minutes.
8. Add water (130–140° F.) to bring the contents to within one-fourth inch of the base of the neck of the bottle.
9. Centrifuge for two minutes.
10. Add enough water (130–140° F.) to keep the fat within the graduated portion of the neck of the bottle until read.
11. Centrifuge one minute.
12. Place the bottles in a water bath at 130° F.
13. Allow a few drops of glymol to run down the inside of the neck of the bottle just before reading. Measure the length of the fat column from the

bottom of the lower meniscus to the sharp line of demarcation between the glymol and the fat.

DIRECTIONS FOR USING THE MINNESOTA METHOD

I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

II. *Reagents*

Minnesota Babcock reagent.

III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk, even though the weight of sample varies. The procedure is as follows as recommended by Thurston and Brown:*

1. Add 15 milliliters of Minnesota reagent.
2. Shake thoroughly.
3. Digest 12 to 15 minutes in a gently boiling water bath, having the bottles in a rack held at least 2 inches above the bottom of the bath.
4. Shake the mixture in the test bottle vigorously at the time when at least half the contents of the bottle have turned dark brown (usually about 2½ minutes after placing them in the water bath).
5. Shake vigorously again about one minute later. (Note: Some care may be necessary when starting to shake the bottles the second time, as the alcohol in the reagent may boil off through the neck of the bottle, taking with it some of the mixture.)
6. Place the tests in a centrifuge and centrifuge them for one-half minute at the speed used for the Babcock test.
7. Add hot water (130–140° F.) to float the butterfat well up into the neck of the test bottle.
8. Centrifuge for one-half minute.
9. Place the tests in a water bath at 133–137° F. and leave for 5 minutes.
10. Just before reading each test, allow colored reading fluid (such as glymol) to flow gently onto the surface of the fat column.
11. Hold the bottles in a level position and read as one would read a Babcock cream test.

REMARKS

Either method can be expected to give reliable results within the accuracy of reading the calibrations on the Babcock test bottle and both are well adapted to the determination of fat in sweetened condensed milk and evaporated milk.

* Ice Cream Field, February 1937.

THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE. II. VITAMIN A *PER SE**

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In previous experiments (8) it was found that the vitamin A requirement of dairy cows for the production of milk fat with maximum vitamin A value was approximately 550,000 units per day when the source of vitamin A in the ration was artificially dried alfalfa hay (carotene). Recent experiments have been completed to determine this daily requirement when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil).

EXPERIMENTAL

The general plan of procedure in all of these experiments has been to decrease the vitamin A potency of the milk secreted by the cows to a low level by feeding vitamin A deficient rations and then determine the number of vitamin A units required daily by the cows to restore the vitamin potency of the milk fat to a high level.

The vitamin A deficient ration was composed of beet pulp, and a grain mixture consisting of white corn, oats, linseed oil meal, bone meal and salt. The source of vitamin A in the repletion rations was vitamin A *per se* (fish liver oil).

Throughout this report vitamin A activity is quantitatively expressed as Sherman-Munsel units.

Experiment I

Two Guernsey cows in the early stage of lactation were used in this trial. The cows were fed the vitamin A deficient ration until the vitamin A potency of their milk fat dropped to a low level (12 units per gram). Beginning at this point each cow was fed vitamin A doses of 25,000, 50,000, 75,000, 100,000, and 200,000 units daily in successive 21 day feeding periods.

Representative samples of milk were collected from each cow during the last two days of each period from which butter samples were prepared for biological assay. Each sample of butter was assayed separately for vitamin A potency.

The results of experiment I are shown in table 1 and figure 1.

Experiment II

Two Guernsey cows were used in the feeding trials of this experiment. After their stores of vitamin A had been depleted to a low level, one cow

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TABLE 1

Showing the vitamin A requirement of dairy cows when vitamin A per se was the source of vitamin A in the ration

Trial 1

Period No.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	
		Cow 521	Cow 535
1	Depletion	12	12
2	25,000	10	12
3	50,000	11	12
4	75,000	15	17
5	100,000	30	30
6	200,000	36	34

* Sherman-Munsel units.

was fed daily vitamin A *per se* in units ranging from 50,000 up to 300,000 and back to 50,000 in successive 21 day feeding periods. The other cow, at the end of depletion period, was started on 300,000 vitamin units daily with descending and then ascending quantities fed in successive feeding periods. The feeding schedule of the two cows is shown in table 2.

DISCUSSION

As has been stated previously (8) the criterion for the measurement of the vitamin A requirements of dairy cows for the secretion of milk fat with maximum vitamin A value is based upon the supposition that cows are not able to secrete butterfat of maximum vitamin A value until the optimum requirements for maintenance and production have been satisfied. Therefore, the minimum vitamin A intake which will produce the maximum effect upon the milk fat secreted should be the minimum vitamin A requirement of the cow for the production of milk fat of maximum vitamin A value.

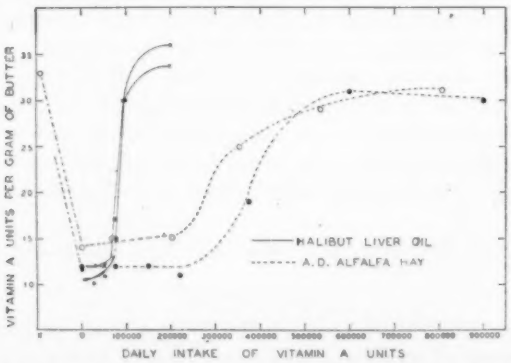

FIG. 1. Showing the relative efficiency of vitamin A *per se* and carotene (artificially dried alfalfa hay) for the production of butter of maximum vitamin A value.

TABLE 2

Showing the vitamin A requirements of dairy cows when vitamin A per se was the source of vitamin A in the ration

Period No.	Trial 2—Cow 534		Trial 3—Cow 535	
	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.
1	Depletion	12	Depletion	15
2	50,000	15	300,000	33
3	75,000	18	200,000	33
4	100,000	24	150,000	30
5	150,000	30	100,000	28
6	200,000	32	75,000	21
7	300,000	35	50,000	14
8	200,000	34	0	14
9	75,000	27	100,000	27
10	0	16	200,000	31

* Sherman-Munsel units.

Previous tests have shown that when dehydrated alfalfa hay was used as a source of vitamin A (carotene) the cows required approximately 550,000 vitamin A units daily to restore the vitamin A potency of the butterfat to its highest level.

In the present tests when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), these requirements were satisfied with approximately 200,000 units daily. For simplicity in showing the relative efficiency of these two sources of vitamin A, the daily unit intake required of each is shown in figure 1. Data for the carotene (dehydrated alfalfa hay) requirements are taken from previous experiments (8).

In trial I, it became evident that the vitamin A requirement for the production of milk of high vitamin A value was between 150,000 and 200,000 units daily when the source of vitamin A in the ration was vitamin A *per se*. In order to check these results and to determine the requirements as affected by depletion and repletion of body storage trials 2 and 3 were conducted.

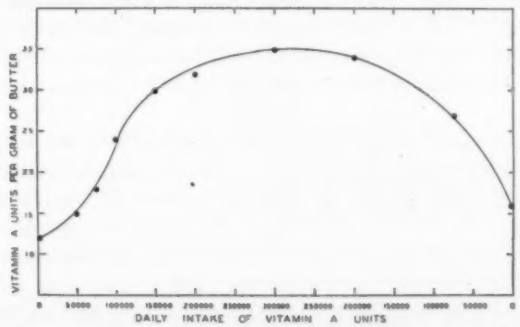

FIG. 2. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value.

As shown in table 2 and figures 2 and 3 the results of the second experiment were almost identical with those of the first experiment. As can be seen in figure 2 when the vitamin A *per se* in the ration was increased from 50,000 to 100,000 units daily there was a significant increase in the vitamin A potency of the milk fat secreted by the cows. The vitamin A value of the milk fat reached its maximum value when the ration contained approximately 200,000 units daily. Increasing the daily vitamin A intake to 300,000 units failed to produce any significant increase in the amount of vitamin A in the milk fat. On the other hand when the amount of vitamin A in the ration was decreased below 200,000 units daily in successive 21-day periods there was a corresponding decrease in the vitamin A potency of the milk fat secreted by the cows. There was, however, a slight lag during the depletion which was probably due to the buffering action of reserve body stores which had accumulated on the high levels.

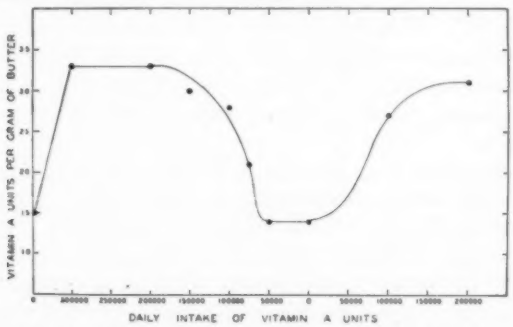

FIG. 3. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value when measured by depletion and repletion tests.

When 300,000 units were fed in the first period following a short depletion period the vitamin A potency of the milk fat was restored to its maximum value immediately. This is shown in table 2 and figure 3. Decreasing the unit intake to 200,000 daily failed to have any significant effect on the vitamin A value of the milk fat. With each increment decrease below 200,000 units daily there was a decrease in the vitamin A potency of the milk fat.

Following the period in which the daily unit intake had been reduced to 50,000 units the cow was again placed on the vitamin A deficient ration for 21 days, during which time the vitamin A potency of the milk fat dropped to a low level (14 units). Following this depletion period 100,000 and 200,000 units was again included in the ration in two successive 21-day periods.

As can be seen in table 2 and figure 3 there was a slight lag in the restoration of the vitamin A potency of the milk fat following this depletion period.

This was probably due to the use of a portion of the vitamin A intake for repletion of body tissues. It should be noted, however, that when 200,000 units were fed daily the vitamin A value of the milk fat again approached its maximum value.

It has been generally recognized that there exists a threshold level for the secretion of vitamin A in milk fat above which more vitamin A or carotene in the feed does not affect the vitamin A potency of butterfat (1, 2, 6, 7). On the other hand, Deuel *et al.* (3) failed to show a definite threshold level in their experiments when they reported that the feeding of shark liver oil produced butterfats of exceptionally high vitamin A potencies as determined by colorimetric and spectrophotometric methods. Rusoff *et al.* (7) reinvestigated this problem and found that feeding 2,144,000 units daily failed to raise the vitamin A potency of the milk above the characteristic threshold level when the vitamin A potency was measured by biological assays. Jensen *et al.* (5), while studying the effect of feeding shark liver oil, found a threshold level in the vitamin A content of blood plasma. The results of our experiments indicate that a definite threshold level for vitamin A does exist. Under the conditions of these experiments, a daily intake of approximately 200,000 units of vitamin A *per se* or 550,000 units of carotene are required to reach this threshold level.

The minimum vitamin A requirement of dairy cows for the secretion of butterfat of maximum vitamin A value has been found to be approximately 200,000 units daily when the source of vitamin A in the diet is vitamin A *per se* and 550,000 units when supplied by carotene in dehydrated alfalfa hay. This would indicate that on the basis of daily unit intake, carotene is only about one-third as effective as vitamin A. Similar differences in effectiveness of these two sources of vitamin A have been reported by Guilbert *et al.* (4) when they studied the minimum carotene and vitamin A requirements of cattle, sheep and swine for the prevention of night blindness.

Although there appears to be a difference in the vitamin A requirements of the dairy cow when vitamin A *per se* or carotene are fed, it is conceivable that the actual physiological requirement is the same. The difference in effectiveness may be due to lower metabolic efficiency in the utilization of carotene or possibly to relative unavailability of the carotene in plant tissue.

SUMMARY

1. Feeding experiments have been completed to determine the minimum vitamin A requirements of dairy cows for the production of butter of maximum vitamin A value.

2. When the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), this requirement was satisfied with a daily intake of 200,000 units.

3. In this series of experiments, vitamin A *per se* was found to be approximately three times as effective as carotene in dehydrated alfalfa hay.

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THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE. III. AVAILABILITY OF CAROTENE IN DEHYDRATED ALFALFA HAY AS COMPARED WITH CAROTENE IN OIL[1]

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In previous reports (4, 9), it has been shown that the apparent vitamin A requirement of dairy cows for the secretion of milk fat with maximum vitamin A value depends upon the source of the vitamin A in the ration. When the source of vitamin A in the ration was carotene from dehydrated alfalfa hay, it was found that the daily requirement was approximately 550,000 Sherman units (9). With vitamin A *per se* this requirement was satisfied with a daily intake of approximately 200,000 units per day (4). Similar differences in the relative efficiency of utilization of carotene and vitamin A by cattle, sheep and swine have been reported by Guilbert *et al.* (2).

Carotene in some plant tissues has been found to be less effective biologically than would be indicated by analyses. Wide variations in the efficiency of utilization of carotene from different plant sources have been reported by Smith and Otis (8) and by Graves (1).

The purpose of this investigation was to compare the availability and efficiency of utilization of crystalline carotene with carotene in dehydrated alfalfa hay for the production of milk fat of high vitamin A value.

EXPERIMENTAL

Two groups of two Guernsey cows each were used in the feeding trials to determine the relative availability of the carotene in dehydrated alfalfa hay as compared with crystalline carotene in oil. The cows were similar in respect to stage of lactation, milk production and body weights. The body stores of vitamin A in the cows were depleted in a preliminary feeding period by feeding a vitamin A deficient ration consisting of beet pulp and a grain mixture of 400 lbs. white corn, 200 lbs. oats, 150 lbs. linseed oil meal, bone meal and salt. In successive feeding periods, the carotene of the hay and the carotene in the oil were equilibrated at levels of 130, 200, 300, and 200 milligrams daily during the respective feeding periods of 21 days each.

The dehydrated alfalfa hay was sacked immediately after dehydration and stored. Prior to each feeding period, sufficient number of sacks of hay

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for the next feeding period were set aside, carefully sampled, and analyzed for carotene. In preliminary determinations of carotene it was observed that the Peterson-Hughes method (6) gave consistently higher values than the methods of Hegsted, Porter and Peterson (3) and Moore (5). Since in each of these procedures a different principle is involved for the removal of the non-carotene chromogens, tests were made to determine the effectiveness of each procedure. Samples of dehydrated alfalfa hay were extracted according to the Peterson-Hughes procedure. The final petroleum ether extracts were washed with water and made to volume. Critical studies upon aliquots from these solutions disclosed that extraction of the non-carotene pigments with 94 per cent diacetone alcohol or by adsorption upon a dicalcium phosphate column were equally effective and gave carotene values in close agreement, while extraction with 90 per cent methanol failed to remove

TABLE 1

A comparison of the relative availability of carotene in dehydrated alfalfa hay and crystalline carotene in oil

Period	Group No.	Carotene supplement*		Butterfat	
		Dehydrated alfalfa hay	Carotene (in oil)	Sample No.	Vitamin A potency
		mg. per day	*mg. per day*		*Sherman units per gram*
1	1	None	None	1	11
	2	None	None	2	13
2	1	130	None	3	19
	2	None	130	4	19
3	1	200	None	5	32
	2	None	200	6	34
4	1	300	None	7	37
	2	None	300	8	36
5	1	None	None	9	22
	2	None	None	10	21
6	1	None	200	11	32
	2	200	None	12	33

* Ration consisted of beet pulp and a grain ration consisting of 400 lbs. white corn, 200 lbs. oats and 150 lbs. linseed oil meal.

some of the pigments and gave high carotene values. When these latter solutions were purified by further treatment with either diacetone or dicalcium phosphate, the carotene values were lowered to agree with the others. Therefore, in these experiments, the carotene values obtained by the Moore procedure (5) were used in calculating the amounts of hay to be fed daily to the cows.

The crystalline carotene in oil was purchased from the National Research Associates, Inc., as a product known as "Research" Carrot Oil which had been prepared from carrots as a thick suspension of crystalline carotene in oil (approximately 3.2 per cent carotene). Upon receipt of the oil, it was removed from the container, thoroughly mixed and bottled in 250-ml. brown bottles under an atmosphere of nitrogen. Samples were taken from a num-

ber of bottles for carotene analyses. These gave concordant results, indicating that the distribution of carotene was uniform. To prevent deterioration, the carrot oil was kept in cold storage until used.

For the carotene analyses, aliquots were dissolved in petroleum ether and the carotene determined with a Bausch and Lomb Spectrophotometer. By passing the petroleum ether solution through a dicalcium phosphate column to remove the non-carotene pigments, it was found to contain 5 per cent impurities. According to the chromatographic analyses which were made by J. W. White, Jr., the carotene was composed of 25.2 per cent alpha-carotene and 74.8 per cent beta-carotene.

Since alpha-carotene possesses but half the biological activity of beta-carotene, it was necessary to equilibrate the carotene of the carrot oil to a biological equivalence of beta-carotene. On the basis of these corrected carotene values, the requisite amount of carrot oil for each feeding dose was weighed into No. 10 gelatin capsules and fed to the cows.

At the end of each feeding period 24-hour composite samples of milk from each group were collected and the cream separated and churned into butter. The butter samples were stored at $-18°$ C. and the portions were removed as needed for biological assays. The butterfat was heated to 55° C. and filtered to remove curd and water. The results of the biological assays of the butterfats are given in table 1.

DISCUSSION

In previous experiments (4, 9), it was found that the carotene of dehydrated alfalfa hay was only about one-third as efficient as vitamin A *per se* as a source of vitamin A in the rations of dairy cows for the production of milk fat of maximum vitamin A value. This difference might be due either to the relative unavailability of carotene in the plant tissue, or if available, to differences in the metabolic efficiencies. A comparison of the availability of crystalline carotene and the carotene in plant tissue should give an insight into this problem.

To test these possibilities, it was imperative that comparisons be made at levels which would be potentially capable of producing differences in the vitamin A potencies of the butterfats. If comparisons were made at too low levels, it is conceivable that differences might exist which would not be reflected in the vitamin A potencies of the butterfat. Likewise, if comparisons were made at levels above the minimum requirements of the cow for the production of butterfat of maximum vitamin A value differences would not be measurable. Therefore, levels of carotene intake were selected which might be expected to be intermediate between these two points.

The results of these experiments, as shown in table 1, indicate that the dairy cow can utilize the carotene in alfalfa hay as readily as isolated carotene for the production of butterfat of high vitamin A value. It is interesting to note that the vitamin A potencies of the butterfats produced by the

two groups on each of the different levels were very similar. This is true even when the two groups were reversed in period 6.

In experiments to determine the minimum carotene requirements of cattle, swine, and sheep for the prevention of night blindness, Guilbert *et al.* (2) found that the requirements were practically the same when the carotene was furnished by alfalfa or by crystalline carotene dissolved in cottonseed oil. In experiments to determine the requirements of chickens for vitamin A when fed as carotene, Sherwood and Fraps (7) found that there was no apparent difference in the effectiveness of carotene supplied in alfalfa meal or by crystalline carotene in oil.

Considering the evidence from all these experiments, it is apparent that the difference in effectiveness of carotene in alfalfa hay and vitamin A *per se* is not due to the unavailability of the carotene in the plant tissue but rather is due to differences in the metabolic efficiencies.

SUMMARY

1. Experiments have been conducted to determine the relative availability of carotene in dehydrated alfalfa hay as compared with isolated carotene (in oil) as a source of vitamin A in the rations of dairy cows.

2. Two groups of cows were used in these experiments. Each group of cows was fed equal amounts of carotene at levels of 130, 200, and 300 milligrams daily.

3. The results of these experiments indicate that dairy cows can utilize the carotene from the alfalfa hay as readily as isolated carotene.

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TEMPERATURE ERRORS IN WEIGHING AND THEIR CONTROL IN THE MOJONNIER FAT TEST

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The Mojonnier test for fat is one of the most accurate methods available for the analysis of dairy products. Nevertheless, analyses of duplicate samples made in different laboratories sometimes differ widely. It is the purpose of this paper to present data concerning one source of error in this test, temperature variations; and to describe an improved method of temperature control for Mojonnier machines.

EXPERIMENTAL

Experiment 1

It is a common observation that the weight of Mojonnier fat dishes can be reduced by warming. This is easily explained. When the dish is warmed, the air in it expands, and a part of it is expelled from the dish as effectively as if a cover had been placed over it and a part of the air had been removed with a vacuum pump. As a result, the dish will appear lighter in weight. In order to avoid such errors, the temperature of the air inside the dish must be the same as that of the air surrounding the dish.

Quantitative data on the relation between temperature and weight were obtained by weighing dishes first at one temperature and then at another. This was accomplished by placing two Mojonnier machines side by side and adjusting the water in one reservoir so that it was about 3° C. above the temperature of the water in the other machine. Four clean fat dishes were placed in the cooling oven of the warmer machine and, after 10 minutes, each dish was weighed. Immediately after weighing, the dishes were placed in the cooler machine. When all of the dishes had been weighed from the warmer machine, they were re-weighed from the cooler machine. This procedure of weighing the dishes first from one machine and then the other was repeated several times. The data are shown in figure 1. The upper line indicates the successive temperatures at which the dishes were equilibrated. The lower line shows the average weight of the four dishes after equilibrating at the temperature shown above. It is evident that a variation of only one degree in temperature may cause a change of approximately $\frac{3}{4}$ mg. in weight. This is equivalent to an error of .07 per cent of fat when testing heavy cream.

Experiment 2

It seemed desirable to find a method of reducing errors caused by improper temperature of the cooling oven. Two procedures were tried. First,

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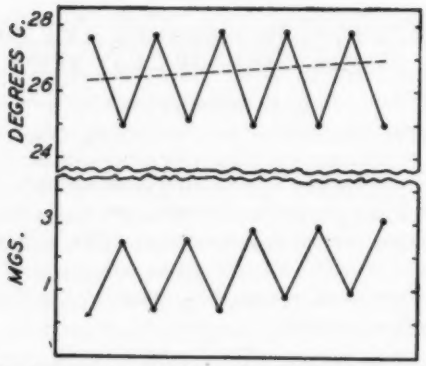

FIG. 1. Variation in the weight of Mojonnier fat dishes (below) due to variation in temperature of the dishes (above). (Actual average weight, 36.0600 grams plus the value shown.)

experiment No. 1 was repeated except that each dish was waved slowly through the air for fifteen seconds immediately before it was weighed. Second, the dishes were removed from the cooling oven and, before weighing, they were placed for two minutes upon a large sheet of $\frac{1}{16}$-inch thick aluminum. This sheet was supported only at the corners so that air could circulate around it freely.

The data are presented in figures 2 and 3. It is evident that both procedures reduced the error due to improper machine temperature, but even the second procedure was not entirely adequate.

Experiment 3

An examination of figure 1 shows that there was a constant difference in weight of about 2 mg., depending upon whether the dishes were weighed

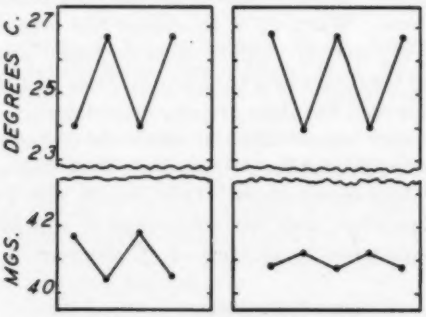

FIG. 2. FIG. 3.

Variation in the weight of Mojonnier fat dishes (below) after tempering in oven at the temperature indicated above. Figure 2, dishes waved in air fifteen seconds before weighing. Figure 3, dishes left on metal plate for two minutes before weighing.

from the cold or the warm machine. It is evident, also, that all the dishes increased in weight in successive weighings at the same temperature. This slow increase in weight was due to a change in the temperature of the laboratory. The actual change in temperature is recorded by the broken line in the upper part of figure 1. It must be remembered that an error in weight may occur if either the temperature of the room, or the temperature of the dishes, is changed.

The following procedure was used to measure the effect of changes in room temperature upon the weight of fat dishes. A set of four dishes was placed in a Mojonnier machine. The water in this machine was held at a constant temperature while the room was warmed slowly by steam radiators. Three electric fans were used to distribute the heat uniformly through all parts of the laboratory. At intervals, the dishes were removed from the machine, weighed at once, and then returned to the cooling oven. The broken line in figure 4 shows the relation between the weight of the dishes

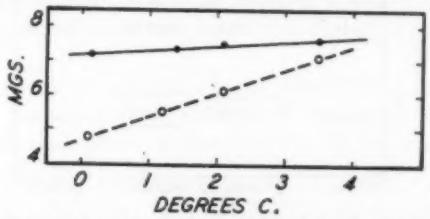

FIG. 4. The relation between the weight of Mojonnier fat dishes and increases in the laboratory temperature. (Machine temperature held constant.) Broken line: weighed direct from cooling oven. Solid line: held on metal plate for two minutes before weighing.

and the increase in the temperature of the room. The water temperature remained constant within 0.1° C.

This experiment was repeated with one change in procedure. Before weighing, each dish was placed on the large aluminum sheet for a period of two minutes. These data are shown by the solid line in figure 4.

Experiment 4

The Mojonnier machine is designed to bring all dishes to the same temperature before they are weighed. Unfortunately, in most cases, this is not room temperature. In those laboratories where the temperature varies considerably during a 24-hour period, this may lead to appreciable errors. If error is to be avoided, the dishes should be brought to the temperature of the balance, regardless of what that temperature may be, before the dishes are weighed. To accomplish this, some means should be provided for maintaining the cooling oven always at room temperature. It would seem much wiser to circulate the cooling water through an air-cooled radiator than to use a large water reservoir in a Mojonnier machine.

This idea was tested by substituting a small radiator from an automobile heater for the water reservoir of one machine. Air was blown over the radiator by an electric fan. A standard Mojonnier machine was used as a control in comparison with this air-cooled machine.

Two sets of clean fat dishes were used, one set for each machine. The experiment was divided into four periods:

1. All dishes were left on top of the balance case until they showed constant weight. This may be considered the true weight of the dishes, free from temperature errors.

2. Each set of dishes was weighed several times from its own cooling oven to get an average value which was reproducible. Since the cooling ovens had not been adjusted to exactly room temperature, these weights differed from the true weights.

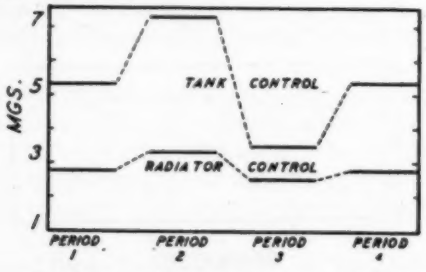

FIG. 5. A comparison of the ability of a tank-controlled machine and a radiator-controlled machine to maintain dishes at constant weight when the room temperature varies. Period 1, dishes at room temperature. Period 2, dishes weighed from cooling oven. Period 3, laboratory cooled 5° C., dishes again weighed from oven. Period 4, dishes at room temperature (5° C. lower than during period 1).

3. The laboratory was cooled 5° C. during a period of 90 minutes. Four electric fans were used to maintain uniform temperature distribution throughout the laboratory. When the room had been cooled, the dishes were weighed again from their respective ovens.

4. The fourth and final set of weighings was made after the dishes had rested upon the top of the balance case long enough to come to the temperature of the room. This set of weighings is comparable with the first except that the laboratory had been cooled 5° C.

The data are presented in figure 5. It is evident that the use of an air-cooled radiator in place of a water reservoir on a Mojonnier machine would be quite helpful in any laboratory which is subject to temperature variations.

Experiment 5

As a further test of the ability of the small radiator to maintain the cooling oven at room temperature, the room was cooled very rapidly, and

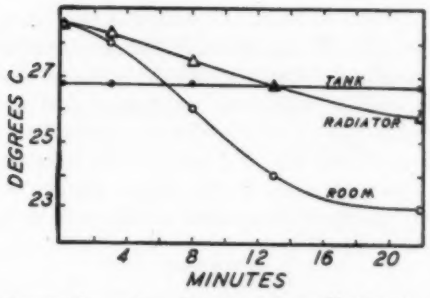

FIG. 6. The comparative rates of temperature adjustment of a tank-controlled machine and a radiator-controlled machine during a period of rapid change in laboratory temperature.

records were kept of the temperature of the room, the temperature of the water circulating through the cooling oven of a standard Mojonnier machine, and the temperature of that circulated through the oven of the air-cooled machine.

The data are shown in figure 6. It is evident that the radiator was too small to keep up with such rapid changes in room temperature though, in this respect, it was far superior to the reservoir on the other machine.

Experiment 6

The magnitude of the error due to improper temperature of the dishes is dependent upon the speed with which they are weighed. If the operator were slow enough, the dishes could come to room temperature while he was adjusting weights. In all of the experiments reported thus far, the actual weighing was performed as rapidly as possible, but no time records were kept.

An experiment was carried out to determine the effect of speed of weighing upon the errors in weight. Dishes were removed from an oven which

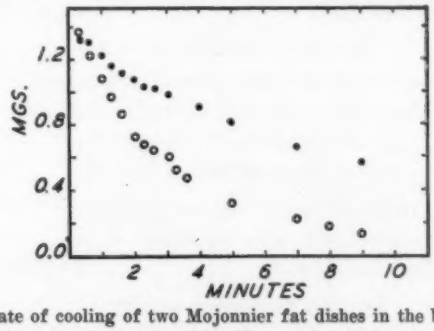

FIG. 7. The rate of cooling of two Mojonnier fat dishes in the balance case as measured by differences between the weight observed at various intervals after removing from the Mojonnier machine and the final equilibrium weight.

was slightly warmer than the room and placed at once upon a balance. Apparent weights were recorded at intervals for thirty minutes, or until the weight remained constant. The final weight was considered the true one, and the errors of earlier weighings were calculated accordingly. Such measurements indicate how slowly the dishes acquire the temperature of the balance case. It was found that different dishes cooled at different rates. Typical data are shown in figure 7. To permit the use of a larger scale in plotting, data are given for only the first ten minutes of the cooling period.

SUMMARY

Errors in Mojonnier fat tests may occur whenever the temperature of the room varies during the course of the analysis. Data are presented showing the magnitude of such errors.

It would be preferable to circulate the water for a Mojonnier machine through an air-cooled radiator instead of through a reservoir. Data are given showing the advantages of such a device.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

1. **Food Enough.** JOHN D. BLACK. The Jaques Cattell Press, Lancaster, Pa. 1943. $2.50.

In addition to including basic facts on the need for food and food nutrients, this book analyzes the world's food supplies and the nutritional status of various peoples, points out the problems of war time feeding, considers production of food on the basis of most effective utilization of natural resources, suggests policies that would best cope with the present food and feed crisis, and recommends a course of marketing procedure designed to overcome much of the difficulties arising between the points of production and ultimate consumption.

Included also are excellent discussions of the need for and mechanics of rationing, shifts in dietary patterns, industrial feeding, and the world-wide significance of the United Nations Conference on Food and Agriculture.

The author is Henry Lee Professor of Economics at Harvard University, a member of the Economic Panel of the Interim Commission of the United Nations Food Conference, and a member of the Food and Nutrition Board of the National Research Council.

The importance of food and the relationships between food supplies and the course of world events now and in the future make this volume of great timeliness. Written by an economist with much practical experience and with many associations that place him in a position to know the facts, and written in a style that is easily read by anyone, *Food Enough* should be on the "must" list for all. Agriculture in general and dairying in particular are given encouraging places in future world economy. W.E.Krauss.

BACTERIOLOGY

2. **Bacteriophages for *Streptococcus cremoris* Phage Development at Various Temperatures.** G. J. E. HUNTER, Dairy Res. Inst., Palmerston North, New Zealand. Jour. Dairy Res., *13*, No. 2: 136–145. 1943.

The effect of temperature on the growth in milk of several strains of *Streptococcus cremoris* and their appropriate phages was investigated. Acid formation and rate of phage multiplication were followed in 100-ml. quantities of autoclaved skimmilk seeded with 1% of an 18–20-hour clotted-milk culture and varying amounts of phage added. At intervals 2 ml. were withdrawn for acidity titration and 1 ml. was used for dilution in the plate test for phage multiplication.

In general, the streptococci grew more rapidly at 30° C. (86° F.) than at 22° C. (71.6° F.) but were inhibited to a greater or less degree at 37° C. (98.6° F.). The phage races for the most part developed somewhat more readily at 30° C. (86.0° F.) than at 22° C. (71.6° F.). Some developed just as readily at 37° C. (98.6° F.) as at 30° C. (86° F.) but others were completely inhibited at 37° C. (98.6° F.).

These results suggest that the phage races as they exist are separate and distinct entities whose growth conditions are similar to, but not necessarily identical with, those of the bacteria upon which they develop. S.T.C.

3. **Distribution of *Flavobacterium maloloris*.** R. M. REYNOLDS AND H. R. THORNTON, Univ. of Alberta, Canada. Sci. Agr., *24*, No. 1: 21. 1943.

Workers in this laboratory had previously found *F. maloloris* to be an infrequent cause for surface taint in butter. In this study 589 isolations of yellow bacteria from 140 samples of Alberta water were examined and only two proved to be *F. maloloris*. O.R.I.

4. **The Occurrence of Slow-Reducing Coliform Organisms in Milk.** C. S. MORRIS, Seale Hayne Agr. Col., Newton Abbott, Devon, England. Jour. Dairy Res., *13*, No. 2: 115–118. 1943.

Milk samples were found frequently to contain coliform organisms which give very slow reduction of methylene blue at 37° C. (98.6° F.). Evidence was secured which indicates that this slow reduction is due to two factors present in raw milk, both of which are destroyed by heating milk to 70° C. (158° F.) for one hour. These factors are (a) a specific bactericidal substance and (b) a growth-inhibiting factor.

All of the cultures examined appeared from differential tests to be intermediate types of coliform organisms. All were citrate negative and failed to produce acid or gas when grown in MacConkey's broth at 44° C. (111.2° F.). S.T.C.

BUTTER

5. **Churn Washing Procedure.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., *5*, No. 2: 120–122. 1943.

Dry rot of staves of the churn barrel is caused by fungi which could be destroyed by heat if it were possible to sufficiently heat all parts, around bolts, crevices, and corners. Cypress is less susceptible to dry rot than Douglas fir. The treatment of churns with hot water apparently neither weakens the wood nor favors dry rot. The most satisfactory procedure of washing the churn appears to be, first, a rinse with water at 120–140° F. to remove the bulk of the fat; second, running the churn for 15 minutes half full of water at 180° F. containing a mild alkaline washing powder; and,

third, running the churn for 15 minutes half full of water at 200° F., draining thoroughly, and allowing to dry with the doors open. Reduction in the temperature of the water does not accomplish the purpose or prolong the life of the churn. P.S.L.

6. **Water Supply Difficulties.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., *5*, No. 2: 46–48. 1943.

Where raw water used as wash water for butter causes off flavors due to bacterial contamination and chlorinated water does the same due to its chemical content, the ideal procedure is to dechlorinate the water before use. The ultra violet ray water sterilizer is of little value for this purpose. The use of small amounts of chlorine causes more off flavor due to chlorophenol and similar compounds than does large amounts of chlorine. Many creameries successfully use wash water containing 25 p.p.m. of chlorine. The writer describes a method for construction of a dechlorinating filter. P.S.L.

7. **Court Cases Involving Butter.** LEO T. PARKER, Attorney at Law, Cincinnati, O. Amer. Butter Rev., *5*, No. 1: 8, 10, 12. 1943.

The author lists several legal interpretations, decisions, and rulings concerning butter; the validity of state laws; public policy, monopoly, trade mark, and ceiling price laws; and validity of contracts. Each is illustrated with recent court decisions. P.S.L.

8. **Moisture Loss in Prints—Combatting Leaky Bodied Butter.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., *5*, No. 3: 86. 1943.

Due to reduced water retaining powers winter butter moisture standards should be reduced if leakiness is to be prevented. Such procedure as reduces size of the water droplets helps in reduction of leakiness. These include working the butter while firm, use of 40° F. wash water, filling the churn no more than 40% full, prevention of stickiness of the churn, and adding all water of standardization at the time of adding the salt. P.S.L.

CHEESE

9. **Bacteria, Environment and Cheese.** E. G. HASTINGS, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., *34*, No. 10: 14. Oct., 1943.

Its environment determines the fate of every living thing—although man cannot always recognize how it does so. The cheesemaker seeks to establish in milk, curd and cheese a desired bacterial pattern by controlling tempera-

ture, acidity, moisture and salt. His sensitivity to the milk is most important in the making of uniform cheese. The uniformity expected by many is unattainable but can be best achieved by the use of milk from the same farms daily, to the bacterial pattern of which the maker has adapted himself. A change of milk or maker can be expected to change quality. Pasteurization of milk simplifies the bacterial pattern and though it slows flavor development it may be possible to find ripening agents to remedy this effect. If normal, three or four months old pasteurized-milk cheese is ground and stirred thoroughly, then flavor development approximates that in raw-milk cheese. The few essential, flavor-producing bacteria which survive pasteurization are scattered by this mixing, so that they reach the food they need and quickly produce flavoring substances. Ground cheese can also be blended for uniformity and reformed and packaged if provision is made for the venting of CO_2 formed by the continued action of ripening agents. Occasionallly pasteurized-milk cheese cures normally without CO_2 development which suggests new ripening possibilities if it were understood. Too little attention has been given to producing the smooth, waxy body desired by most consumers; too much attention has been given to eye formation in Swiss. "Eyes in a woman or a Swiss cheese are something to consider but not to control one's judgment as to desirability." W.V.P.

10. The Effect of Over-Ripening upon the Activity of Cheddar Cheese Starters. C. K. JOHNS AND H. L. BERARD, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. Jour. Dairy Res., *13*, No. 2: 127–135. 1943.

In a study involving three starters, over-ripening brought about by increasing the amount of inoculum, by lengthening the period of incubation, or by increasing the temperature of incubation, failed to slow down the rate of bacterial growth, or acid development, or to lower the final acidity reached. Starters repeatedly over-ripened, over a period of 30 days were considered superior in flavor to those normally ripened.

In practical cheesemaking an over-ripened portion of a starter worked slightly faster in the vat and produced cheese with a higher flavor score than the control. S.T.C.

11. The Influence of Abnormal ("Non-Acid") Milk on Cheese Starter Cultures. G. J. E. HUNTER AND H. R. WHITEHEAD, Dairy Res. Inst., Palmerston North, New Zealand. Jour. Dairy Res., *13*, No. 2: 123–126. 1943.

"Non-acid" milk is described as a colloquial term coined by cheesemakers in New Zealand to designate milk which hinders the development of acid by a normally active starter culture in the cheese vat. Stock cultures of "non-acid" organisms were grown at 30° C. (86° F.) in sterilized skimmilk for

24 hours. The acidity rise was 0.03–0.06 per cent expressed as lactic acid. This "non-acid" skimmilk was mixed in varying proportions with normal fresh milk and the resulting mixture used for starter propagation. Delayed coagulation simulating a starter failure caused by bacteriophage was demonstrated. Some strains of starter cultures were considerably less inhibited than others. S.T.C.

12. **Further Studies on Bacteriophage in Relation to Cheddar Cheesemaking.** C. K. JOHNS, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. Jour. Dairy Res., *13*, No. 2: 119–122. 1943.

Trouble with slow working vats in two factories which had continued over a period of six weeks was shown to be due to lysis of the dominant strains of the mixed culture of starter organisms by phage. Normal acid development was secured by substitution of a starter of entirely different bacterial strains.

A second outbreak of phage infection with complete cessation of acid production in experimental vats is described. The starter itself appeared to be entirely free from phage and the equipment had been drastically sterilized following a previous outbreak. The original source of the phage was not definitely demonstrated although occasional positive indications were obtained from the milk supply itself. S.T.C.

13. **The Production of Rennet from Living Calves.** N. J. BERRIDGE, J. G. DAVIS, P. M. KON, S. K. KON, AND F. R. SPRATLING, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., *13*, No. 2: 145–161. 1943.

Abomasal juice containing rennin was obtained from living calves by means of an abomasal fistula. The operation for fistula was performed on two calves at 14 days of age. The animals received, during the three months for which the experiment lasted, an exclusive diet of whole milk supplemented by minerals and vitamin D.

Abomasal juice was obtained by allowing the calves to drink dilute whey and removing it through the fistula in about half an hour. The mean yield of rennet for each "perfusion" from the first calf was 3120 units with a standard deviation of $\pm$ 1330 and 5680 units with a standard deviation of $\pm$ 2560 from the second calf. One unit of rennet is defined as the amount sufficient to coagulate in 100 seconds 10 ml. of a substrate consisting of 12 grams of spray-dried skimmilk in 100 ml. of N/50 calcium chloride solution.

A concentrated rennet was prepared from the abomasal juice. Cheese made from the fistula rennet was indistinguishable from the control cheese.

This method of rennet production was regarded as too expensive in time, labor, and cost of food for use in commercial rennet production in England. S.T.C.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

14. Relation of Lecithin to the Keeping Quality of Dry Whole Milk. C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State College. Natl. Butter and Cheese Jour., *34*, No. 10: 18. Oct., 1943.

Fresh milk was separated into cream and skim milk. The cream was churned and the butter melted and washed to obtain pure butter oil. A portion of the skim was centrifuged at 25,000 RPM to reduce the lecithin in the skim. Samples of the fresh milk, pure butter oil and skim, and pure butter oil and centrifuged skim were pasteurized, homogenized, concentrated 2 to 1, and dried by the atmospheric roll process. The dry milk was placed in brown bottles. Half of each lot was charged with nitrogen, and all containers were carefully sealed and stored at 85° F. Some of each was reconstituted and examined at monthly intervals. The samples with least lecithin kept best although all samples deteriorated. Over half of the milk lecithin remained in the skim milk and though centrifuging reduced the amount, it may also have removed some other pro-oxidant. The experiments indicate the possibility of producing dry milk with excellent keeping qualities. W.V.P.

15. The Gas-Packing and Storage of Milk Powder. C. H. LEA AND T. MORAN, Low Temp. Res. Sta., Cambridge, England, and J. A. B. SMITH, Hannah Dairy Res. Inst., Ayr. Jour. Dairy Res., *13*, No. 2: 162–215. 1943.

The authors present the following summary of their extensive work:

"(a) Full-cream powders stored in the presence of up to 0.01 ml. of oxygen per g. of powder kept very well at both normal and high temperatures. This figure corresponds to 1% oxygen (after completion of desorption) in the free-space gas of a can of spray-dried powder packed to a bulk density of 0.55 g./ml., or to 0.5% of oxygen in a can of roller powder packed to a bulk density of 0.35 g./ml. Tallowiness was never definitely detected under such conditions, and there seems to be little or no advantage to be gained, at least so far as palatability is concerned, by improving on this figure. An atmosphere containing not more than 0.01 ml. of oxygen per g. of powder can therefore be considered an ideal pack for milk powder.

"(b) Powders stored in the presence of 0.02 ml. of oxygen per g., *i.e.*, approximately 2% of oxygen in the free-space gas of a can of spray-dried full-cream powder, also kept well, in fact when storage was at 15° C. (59° F.) it was often impossible to distinguish between such powder and others stored in the presence of much smaller amounts of oxygen. At the high temperature at 37° C. (98.6° F.) the powder was usually, but not invariably distinguishable from powders stored in lower concentrations of oxygen, but the difference was slight. Below 0.02 ml. of oxygen per g. of powder may therefore be considered a good commercial pack.

"(c) Powder stored in the presence of 0.03 ml. of oxygen per g., *i.e.*, approximately 3% of oxygen in the free-space gas of a can of spray-dried full-cream powder, kept quite well. The testing panel could usually distinguish between powder stored for long periods at 15° C. (59° F.) in this and in lower concentrations of oxygen, but the difference was still small and probably not important from a practical point of view. In view of the difficulty of gas-packing, spray-dried powders in very low concentrations of oxygen, values up to 0.03 ml. of oxygen per g. of powder can be accepted as satisfactory for a commercial pack. It should be possible to store full-cream powder under those conditions for several years without serious deterioration. A rather lower concentration of oxygen than 0.03 ml./g. might, however, be desirable if storage is to include exposure to high atmospheric temperatures.

"(d) Powder stored in the presence of 0.04 ml. of oxygen per g., *i.e.*, approximately 4% oxygen in the free-space of a can of spray-dried full-cream powder, developed a suspicion of 'off' flavor (mark 1.0) in about 7 months and a definite slight 'tallowy' odor and flavor ('off' flavor mark 1.5–2.0) in about 12 months at 15° C. (59° F.), but was little, if any, worse after 2 or 3 years' storage. Though always considered usable and sometimes quite good, such powder was definitely inferior to samples stored in lower concentrations of oxygen, and so high a figure as 0.04 ml./g. should not be accepted as satisfactory. At 37° C. (98.6° F.) results were more unfavorable than at 15° C. (59° F.) and a sample of spray full-cream powder stored at this temperature was considered to have become unusable for part of the storage period, between 4 and 12 months, when 'off' flavor marks between 2.0 and 2.8 were recorded. The powder subsequently improved slightly to mark 2.0 at 12 and 16 months." S.T.C.

DISEASE

16. Variations in the Occurrence of Bloat in the Steer Progeny of Beef Bulls. B. Knapp, Jr., A. L. Baker and R. W. Phillips, U. S. D. A., Beltsville, Md. Jour. Anim. Sci., *2*, No. 3: 221–225. Aug., 1943.

The progeny of 13 registered Hereford bulls were used over a two-year period in this study. A highly significant difference existed between progeny groups in both years on the frequency of bloat. The results indicate that there are inherent differences between progeny of different bulls in ability to handle large quantities of feed without digestive disturbances and indicate the possibility of improvement in this characteristic by selection on the basis of progeny tests. C.F.H.

FEEDS AND FEEDING

17. Determinations of Metabolizable Energy of Feeding Stuffs for Cattle. E. B. Forbes and E. J. Thacker, Dept. Anim. Nutr.,

Pennsylvania State Col., State College, Pa. Jour. Anim. Sci., *2*, No. 3: 226–230. Aug., 1943.

In working out a method for calculating metabolizable energy from the digestible nutrients, a comparison was made between experimentally determined metabolizable energy of a group of feeding stuffs and computed values according to the formula of (1) Bratzler, (2) Kriss and (3) Axelsson. Metabolizable energy values calculated according to the method of Axelsson were the most satisfactory for feeds other than silage. When the factor 3.3 was used in computing metabolizable energy of the digestible ether extract of silage along with Axelsson's factors for digestible protein and carbohydrates of roughages, total metabolizable energy, values of silages were in approximate agreement with values based entirely on experimental observations. C.F.H.

18. Die Eignung von Kartoffeleiweisspülpe als Milchviehkraftfutter. J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, *18*, No. 2: 43–49. Feb., 1943.

Two groups of six cows each were fed 3 kg. per day of this protein pulp to replace an oil cake mixture. The results were unfavorable. This was repeated in another three-week experimental period. When the experiment was again repeated but with only 2 kg. per day, practically no difference between the two groups was obtained. It is tentatively concluded that this feed may be fed with good results if the rate is not in excess of 2 kg. per day. J.L.L.

19. Untersuchungen über die Verwertung von Harnstoffstickstoff durch wachsende Kälber. J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, *18*, No. 1: 1–11. Jan., 1943.

Whether the nitrogen in urea could be used by growing calves to replace part of their protein needs was studied on two pairs of identical twin calves and in another experiment with three lots of five calves each. The first pair of twins was fed for 728 days. The data are presented for them in five consecutive periods. The second pair was fed for 436 days and the results are presented separately for four periods of this time. The three groups were fed over a period of 252 days. The basic ration was clover hay and dried beet pulp. The control groups received in addition a concentrate mixture thought adequate to cover their protein needs. The amounts of digestible crude protein ingested per day were the same, but the digestible true protein was 390 g. per day for the control group and only 190 g. per day for the amide group. The third group was given only as much digestible true protein as the amide group and barely over half as much digestible crude protein. The growth curves of the control group were far above those

of the other two. The growth curve of the group receiving the amide nitrogen was only a little above that of the group which received the same amount of true protein but no extra amide nitrogen. The authors conclude that both sets of experiments show that growing calves are not able to use the nitrogen of urea to any practical extent for growth. This agrees with their earlier and more extensive experiments on sheep. J.L.L.

FOOD VALUE OF DAIRY PRODUCTS

20. Reviews of the Progress of Dairy Science. Section D. The Nutritional Value of Milk and Milk Products. S. K. KON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., *13*, No. 2: 216–241. 1943.

This is an excellent review of the literature on the nutritional value of milk and milk products covering the period from the beginning of 1940 to the end of 1942. 430 references. S.T.C.

ICE CREAM

21. Dried Whole Egg Powder. V. Definition and Properties of Low Grade Powders. M. W. THISTLE, MARGARET REID AND N. E. GIBBONS, Natl. Res. Council of Canada, Ottawa. Canad. Jour. Res., *D*, *21*, No. 8: 267. 1943.

The point at which 50% of a panel of tasters regarded dried egg powder as unsuitable for human consumption coincided with a rating of 2.7 on a scale ranging from 10 for excellent, fresh egg, to 0 for repulsive material. The protein fraction of these low grade samples had deteriorated badly, as shown by fluorescence measurements. The fat fraction showed no evidence of peroxide oxygen formation however. O.R.I.

22. Better Ices and Sherbets. C. D. DAHLE, Pennsylvania State Col., State College, Pa. Ice Cream Trade Jour., *39*, No. 10: 30. Oct., 1943.

The author outlines the requirements for, and characteristics of, high quality sherbets and ices, pointing out the difficulties encountered in manufacture under present conditions. Particularly stressed are the necessity for controlling the overrun under 50%, preferably at about 35%; the use of corn sugars high in dextrin and such cereal products as oat and wheat flour for improving body and texture; the proper amount of sweetening from the standpoint of body and texture as well as flavor; and the necessity of considering the sugar content of fruit products used as flavors. Defects of ices and sherbets are also briefly considered. F.J.D.

23. Wartime Trends in the Retail Ice Cream Store. CHARLES PAINO, Natl. Assoc. Retail Ice Cream Mfgrs. Ice Cream Trade Jour., *39*, No. 10: 26. Oct., 1943.

Results of a survey conducted among 80 companies representing 300 retail ice cream stores revealed that sugar and labor are the most important problems facing operators of stores. Only 2% of the stores have discontinued the sale of cones and in these cases only intermittently. Eighty per cent have discontinued the sale of bulk carry-out ice cream and the other 20% have done so at times, but only 2% have discontinued the sale of packages. Sales of sherbet have ranged from 10% to 50% of the total gallonage with the big majority reporting about 25%. Over half the stores report that the consumer does not like to be forced to buy sherbet with ice cream. All of the stores are confident that they will be able to continue in business during the war. F.J.D.

24. Possible Conversion of Ice Cream Plants to Frozen Foods. F. L. THOMSEN AND RICHARD GABEL, Bur. Agr. Economics, U. S. D. A., Washington, D. C. Ice Cream Trade Jour., *39*, No. 10: 38. Oct., 1943.

This article is the first report of a survey being conducted with the object of determining the practicability of utilizing the resources of the ice cream industry for the freezing and distribution of frozen fruits, vegetables, meats and fish should future conditions in the canning industry demand it. The possibilities of a shortage of tin, should the war with Japan be long drawn out, are pointed out as are also the lack of refrigerator cars and the fact that greater utilization of fresh products is difficult. Some arguments for conversion of a small proportion of the ice cream industries' facilities at an early date rather than a substantial conversion at a later date are advanced. The general theme of the article seems to be that it would be better to play safe by conserving the available tin supply in some such manner rather than to hope for the best and be left high and dry with no tin for even the essential uses. F.J.D.

MILK

25. Dipper Strainer—Flaky Milk. H. J. BRUECKNER, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *5*, No. 1: 10. 1943.

The dipper-strainer, with fine mesh screen bottom, is being used rather successfully at milk stations for detection of mastitis milk. Flakes of fat may be distinguished from curd flakes, and are caused by improper handling of milk at the farm, chiefly by air cooling or slow cooling, mixing warm with cooled milk, and cooling milk in tanks not sufficiently filled with water to

reach the surface of the milk in the can. This method for examination of milk gives promise of much wider adoption. P.S.L.

26. Prospects for Milk Supplies in 1944. T. G. STITTS, Food Distrib. Admin., Washington, D. C. Ice Cream Trade Jour., *39*, No. 10: 22. Oct., 1943.

Indications are that milk production in 1943 will be slightly less than in 1942 and estimations for 1944 are less than for 1943. Serious shortages of butter, cheese and fluid milk have developed in many areas. These are attributed to increase in the consumption of fluid milk and the greater utilization of dairy products by the armed forces and for Lease-Lend purposes. Recent Order No. 79 aims to prevent further increase in the consumption of fluid milk in order that supplies will be available for butter, cheese, and concentrated products. Notwithstanding Order No. 8 which reduced the milk solids going into ice cream by 35%, the total gallonage of frozen dairy foods for 1943 will be no less than in 1942, although it is estimated that the equivalent of 100,000,000 pounds of butter and 60,000,000 pounds of dry skimmilk will have been saved.

The outlook for 1944 is that milk and dairy products will fall short of supplying demands. Subsidies of 25 to 50 cents per cwt. of milk to farmers and 3 to 6 cents per pound of fat for cream have been authorized in an effort to stimulate production.

No immediate change in the quota of milk solids for ice cream purposes is contemplated, but should the demand for milk solids for other, more urgently needed, dairy products become increasingly unsatisfied a review of existing food orders affecting dairy products would become necessary.

F.J.D.

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Sugar—

HOW COULD ANYTHING SO FAMILIAR BE ARTIFICIAL?

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The consumer has little realization of the processing steps required to put sugar on his pantry shelf or delicious ice cream in the cabinet. Yet, the thirst for knowledge about every-day things, offers opportunity for food processors to arouse extra interest in their work. Most people are surprised and interested to learn that food processing requires the taking away of unwanted parts of "natural" products in some cases—adding ingredients in other cases—treating with heat, refrigeration or brine in still other cases.

The behind-the-scenes story about sugar is the romance of a product where Man's interest in one ingredient calls for "processing out" the unwanted parts of Nature's raw product.

In India, which is believed to be the original home of the sugar cane (dating back some 3000 years), the processor eliminates 19 tons of the original cane in order to get 1 ton of sugar from his acre.

The continual selection and breeding of improved varieties of cane, plus the work of capable cultivators and expert processing makes it possible, in other lands, to secure 150 tons of cane from a single acre—which in turn yield 15 tons of sugar.

Where the jungle grass, known as sugar cane, is the source of sugar, the harvested crop starts the processor's work. After the stalks are mangled and milled in the first step to force them to yield their sucrose, the processor finds himself with a dark, opaque liquid. Now, the chemist takes a hand. The liquid contains glucose, fructose, nitrogen compounds, pectin, gum, acids, coloring matters, vanillin, minerals and foreign matter. But even stranger to the layman than this is the news that in order to get just the product wanted, the processor uses in the mixture such chemicals as quick-lime, sulphur dioxide gas, carbon dioxide gas and, on occasion, phosphoric acid. By the time the processor finishes his intricate work of redissolving, clarifying, decolorizing and recrystallizing, the cane sugar that sweetens your coffee is 99.9% pure sucrose.

Nature presents her bounty in a wide variety of ways. In some cases, Man need only pick the finished fruit from a wild tree, but more often the gifts are hidden away and Man must use his ingenuity and effort to develop the finished product from one or many sources.

Sugar is one of Nature's hidden delights. Vanilla flavoring is even more obscure. In addition to the fact that the vanilla bean has to be cured before any semblance of vanilla flavoring appears, Nature set up another treasure hunt to test the processor's skill. The added vanillin required to round out the vanillin and other flavoring constituents of the vanilla bean, is hidden away in the clove spice and other of Nature's products.

So, while sugar is an example of food processing where something *must be taken away* from Nature's offering to produce the desired result, vanilla flavoring is an example of food processing where something *must be added* to produce the best product.

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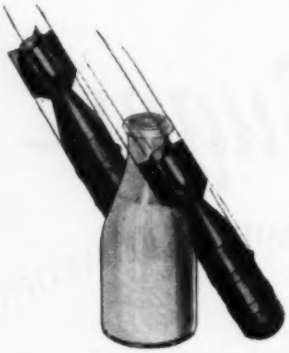

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Sometimes, better formulas are written with an eraser!

Most great products are the result of ceaseless experiment, improvement and change. Yet, how often is all chance of improvement brushed aside with the casual remark—"We don't like to change our formula"? Too frequently, the same old formula has been used so long that even the idea of changing it is seldom considered. But "the same old formula" isn't always the best formula. Vanilla, for example, is such an important ingredient and plays such a big part in the ultimate popularity and sale of any product that nothing less than the best can ever be good enough. Yet, how long is it since you've even questioned the vanilla you are now using?

If you are not one of the many ice cream manufacturers already using Veritivan, why don't you try it and see how much rich, delicious goodness it will add to your own ice cream? We think you'll agree with so many others that Veritivan is truly the best of all fine flavors.

FOOD MATERIALS

CORPORATION

Manufacturing Flavor Chemists

2521 WEST 48TH STREET • CHICAGO

Branch Offices in all Principal Cities

VERITIVAN

Food Materials Corp.
2521 W. 48th St., Chicago, Ill.

Please send 1 lb. Free Sample of Veritivan

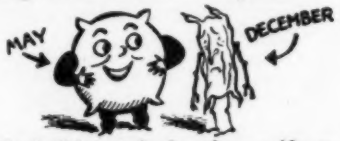
Company_____

By_____

City_____ State_____

"Whad'ya Know, Brother, About BULK?"

EVERY TECHNICAL man is supposed to know his own field thoroughly and have a general idea of related technical fields. But few food processors have time to develop more than a sketchy knowledge of these related subjects.



Take the bulkiness of salt—the specific gravity—which may vary rather widely. A survey of various salt products from different sections of the country has shown that even salt of the same grade can vary up to 25% in bulkiness over a period of months.

Diamond Crystal processing engineers have eliminated this variation to a considerable extent through rigid control of crystallizing conditions and the most modern screening methods. Thus the various grades of Diamond Crystal Salt will not vary more than 4% plus or minus—usually not over 2%.

If you, as a food processor, are applying salt by volume—through a hopper, for example, as in salting soda crackers, potato chips, etc.—you can only achieve flavor control by using a salt that varies little in bulkiness. Why not check the density of each shipment of salt you are using and see how much it varies in bulk?

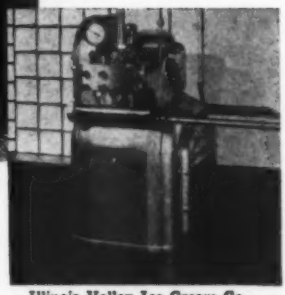
NEED HELP? HERE IT IS!

If this is the solution to that variable-flavor problem, write our Technical Director. He will be glad to help you find a cure. Diamond Crystal, Dept. H-5, St. Clair, Michigan.

DIAMOND CRYSTAL
ALBERGER PROCESS **SALT**

Bowman Dairy Co.—Chicago-River Forest Plant

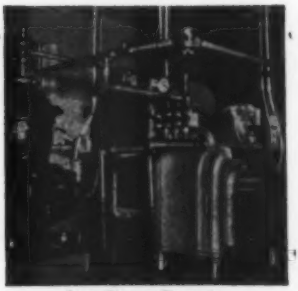
C P
Multi-Flo
Homogenizer



Illinois Valley Ice Cream Co.,
Streator, Ill.

Multi-Flo Homogenizer

. . . A Distinct Advance in Homogenization Efficiency



Star Cheese Factory,
Langlois, Ore.

The CP Multi-Flo is not just another homogenizer. Installations from coast-to-coast prove that it accomplishes a better job with less pressure. Write today for full details on the unique CP Single-Service Multi-Flo Valve . . . the new head construction that cuts disassembling time 50%; plus many other CP design advantages.

A Pet Milk Co. Plant

Creamery Package
Fort Atkinson
Plant

THE CREAMERY PACKAGE MFG. CO.

1243 W. Washington Blvd., Chicago 7, Illinois

Branches: Atlanta — Boston — Buffalo — Chicago — Dallas — Denver — Kansas City — Los Angeles — Minneapolis — New York — Omaha — Philadelphia — Portland, Oregon — Salt Lake City — San Francisco — Seattle — Toledo — Waterloo, Iowa

CREAMERY PACKAGE MFG. CO. OF CANADA, LTD.
267 King St., West, Toronto 2, Ont., Canada

Your advertisement is being read in every State and in 25 Foreign Countries

DIFCO

CULTURE MEDIA
for Examination of Milk

Bacto-Tryptone Glucose Extract Agar

is recommended for use in determining the total bacterial plate count of milk in accordance with the procedures of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association.

Upon plates of medium prepared from Bacto-Tryptone Glucose Extract Agar colonies of the bacteria occurring in milk are larger and more representative than those on media previously used for milk counts.

Bacto-Proteose Tryptone Agar

is recommended for use in determining the bacterial plate count of Certified Milk. The formula for this medium corresponds with that suggested in "Methods and Standards of Certified Milk" of the American Association of Medical Milk Commissions.

Bacto-Violet Red Bile Agar

is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

Bacto-Brilliant Green Bile 2% and
Bacto-Formate Ricinoleate Broth

are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN